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Full Name of Author — Nom complet de l'auteur

John A. Basarab

Date of Birth — Date de naissance Nov. 3/53	Country of Birth — Lieu de naissance Canada

Permanent Address — Résidence fixe

#302, 10524 - 78ave
Edmonton, Alta
Canada T6E 1P4

Title of Thesis — Titre de la thèse

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University of Alberta

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GENETIC AND PHYSIOLOGICAL CHARACTERIZATION OF THE DOUBLE MUSCLED SYNDROME IN CATTLE

by

© JOHN A. BASARAB

A THESIS

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The undersigned certify that they have read, and recommend to the Faculty of Graduate Studies and Research, for acceptance, a thesis entitled GENETIC AND PHYSIOLOGICAL CHARACTERIZATION OF THE DOUBLE MUSCLED SYNDROME IN CATTLE submitted by JOHN A. BASARAB in partial fulfilment of the requirements for the degree of DOCTOR OF PHILOSOPHY in ANIMAL GENETICS AND BIOCHEMISTRY.

R. T. Berg
Supervisor

J. R. Thompson

Fred [illegible]

Robert T. Hardin

H. J. Swatland
External Examiner

Date Aug 17/81

ABSTRACT

A series of studies was conducted to characterize and elucidate the manifestations of the Double Muscled Syndrome in cattle.

In the first study (Chapter I) the breeding history and the phenotypic expression of the "double-muscling" trait in a "Double-Muscled" (DM) population were examined. The evidence suggested that the "double-muscling" trait is mediated by a single recessive gene pair with occasional intermediate expression in the heterozygote mediated by modifying gene-products.

In the second study (Chapter II) the erythrocyte osmotic fragility of nine extreme-muscled DM cattle, 13 carriers of "double-muscling" and 73 normal-muscled cattle of three breed groups were determined. Extreme-muscled DM cattle had an increased ($P<0.05$) erythrocyte osmotic fragility as compared to carriers and normal cattle. Carriers demonstrated an erythrocyte fragility similar to those in two of the normal cattle breed groups.

In study three (Chapter III) the serum triiodothyronine (T_3) and thyroxine (T_4) concentrations in five extreme-muscled DM cattle, 39 carriers and 43 normal cattle were determined. Extreme-muscled DM cattle demonstrated a higher ($P<0.05$) serum T_3 concentration as compared to normal cattle. Carriers of "double-muscling" were intermediate in their serum T_3 concentration. Serum T_4 concentrations were not significantly different between extreme-muscled DM

cattle, carriers and normal cattle.

In the fourth study (Chapter IV) the lipid composition of erythrocyte membranes was determined in 11 extreme-muscled DM cattle and 11 normal-muscled carriers. The concentration of cholesterol and of the different phospholipid classes in erythrocyte membranes from extreme DM cattle and carriers were similar. Extreme-muscled DM cattle demonstrated significant ($P<0.05$) alterations in the fatty acid composition of their erythrocyte membrane sphingomyelin and phosphatidylethanolamine fractions relative to normal-muscled carriers. The most notable differences were in the increased ($P<0.05$) concentration of palmitic acid and decreased ($P<0.01$) concentration of oleic acid in the erythrocyte membrane sphingomyelin fraction from extreme-muscled DM cattle relative to normal-muscled carriers.

The lipid composition of plasma and high density lipoprotein (HDL) and low plus very low density lipoprotein (LDL-VLDL) fractions in six extreme-muscled DM cattle and six normal-muscled carriers were examined in study five (Chapter V). Extreme-muscled DM cattle demonstrated a decreased ($P<0.05$) concentration of triacylglyceride in the plasma, HDL and LDL-VLDL total lipid fractions as compared to normal-muscled carriers. Extreme DM cattle also had an increased ($P<0.05$) concentration of polyunsaturated fatty acids in the plasma and LDL-VLDL total lipid fractions, with a similar trend ($P<0.1$) occurring in the HDL total lipid

fraction, relative to normal-muscled carriers.

In the sixth study (Chapter VI), the *in vitro* glucose consumption and lactic acid production by erythrocytes from 10 extreme-muscled DM cattle, 15 carriers and 20 normal cattle were determined. Erythrocyte glucose consumption and lactic acid production was greater ($P<0.01$) in both extreme-muscled DM cattle and carriers than in normal cattle. Extreme DM cattle were not significantly different ($P>0.05$) in either their erythrocyte glucose consumption or lactic acid production relative to carriers. No difference ($P>0.05$) was observed in the erythrocyte lactic acid production to glucose consumption molar ratio between extreme DM cattle, carriers and normal cattle.

The results of the present series of studies suggest that the primary alteration caused by the gene or genes for "double-muscling" may be in either a structural or enzymatic component of the cell membrane or may reflect an alteration in cell metabolism, possibly resulting from endocrine or neuroendocrine abnormalities in cattle exhibiting the Double Muscled Syndrome.

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GENERAL INTRODUCTION

A major goal in beef cattle breeding and selection is to produce cattle with increased muscling. For this reason, attention has been focused on cattle exhibiting the Double Muscled Syndrome (DMS) or double-muscling (DM) as the condition is commonly known. The Double Muscled Syndrome in cattle is an inherited developmental abnormality affecting skeletal muscle growth and is manifested by an increased potential for muscling (Oliver and Cartwright 1968; Holmes and Ashmore 1972; Hendricks *et al*. 1973; Swatland 1973; Ashmore *et al*. 1974; Swatland and Kieffer 1974; West 1974) and a decreased potential to deposit fat (Oliver and Cartwright 1968). The mode of inheritance of double-muscling in cattle has been described as involving a single recessive gene pair with incomplete penetrance showing variable phenotypic expression in the heterozygote (Oliver and Cartwright 1968; Kieffer *et al*. 1972a, b; Rollins *et al*. 1972; Kieffer and Cartwright 1980).

As the term syndrome implies, "double-muscling" in cattle is also associated with many physical, physiological, histological, histochemical and biochemical characteristics other than the "double-muscling" trait (Lawrie *et al*. 1964; Oliver and Cartwright 1968; Holmes and Ashmore 1972; Holmes *et al*. 1972a, b, c, 1973; Hendricks *et al*. 1973; Ashmore *et al*. 1974; West 1974; King *et al*. 1976a; Carroll *et al*. 1978; Basarab *et al*. 1980; Strath *et al*. 1980). The number, type and intensity of these associated characteristics vary, considerably, depending on the degree of phenotypic expression of the "double-muscling" conformation. This variation has made difficult the complete characterization of the syndrome and the elucidation of the biochemical alteration responsible.

Much of the research done in regard to the Double Muscled Syndrome in cattle has centered around characterization of muscle type (Lawrie *et al*. 1964; Holmes and Ashmore 1972; Hendricks *et al*. 1973; Swatland 1973; Ashmore *et al*. 1974; Swatland and Kieffer 1974; West 1974; Carroll *et al*. 1978), physiological and biochemical response to stressors imposed on these animal types (Ashmore and Robinson 1969; Holmes and Robinson 1970; Holmes *et al*. 1972a, b, c, 1973; Monin and Boccard 1974; Strath *et al*. 1980) and the mode of inheritance of the syndrome (Oliver and Cartwright 1968; Kieffer *et al*. 1972a, b; Rollins *et al*. 1972; Kieffer and Cartwright 1980). An aspect of the Double Muscled Syndrome not yet examined in any detail is the suggestion by King and associates (King and Basrur 1974; King 1975; King *et al*. 1976a) that this condition may be associated with a generalized cell membrane defect.

A series of investigations were therefore undertaken to verify the occurrence of a membrane defect and to elucidate the nature and manifestations of the membrane defect in cattle exhibiting the Double Muscled Syndrome.

I. ORIGIN AND INHERITANCE OF THE "DOUBLE-MUSCLING" CONFORMATION IN THE KINSELLA DM POPULATION

A. Abstract

In 1967 a heavily muscled or Double-Muscled (DM) population was initiated at The University of Alberta Beef Research Ranch, Kinsella, Alberta. Three extreme-muscled DM bulls obtained from outside sources and one extreme-muscled bull from the Kinsella Beef Synthetic (SY) population contributed to the introduction of the "double-muscling" conformation into the present DM herd. Initially, cows bred to these extreme-muscled DM bulls were normal-muscled Angus, Charolais and Galloway cross-breds chosen from the SY population. Since 1976, the DM herd has been bred as a closed breeding population, though, this population is open to extreme-muscled animals which arise in the other Kinsella herds. In 1981, the average breed percentages of the calves born to this herd were Angus 51%, Galloway 14%, Hereford 13%, Charolais 12% and Limousin 8%.

Examination of the breeding history and of the phenotypic expression of the "double-muscling" trait in the DM population suggests that the mode of inheritance of the "double-muscling" trait is controlled by a single recessive gene pair with variable expression in the heterozygote mediated by modifying gene-products. A 2.3:1 male to female sex ratio was observed in extreme-muscled DM offspring suggesting a prenatal selection against extreme-muscled DM

females.

B. Introduction

In recent years a "Double-Muscled" (DM) population has been developed at The University of Alberta Beef Research Ranch, Kinsella, Alberta . Animals from this population have been used in the past, and are at present being used, to study possible abnormalities in membrane function, lipid metabolism and deposition, protein metabolism, stress susceptibility and muscle growth and development which may be manifestations of the Double Muscled Syndrome. An understanding of the animal types present in this population is necessary. This chapter will therefore provide further information about the origin of the "double-muscling" conformation, the breed composition and the genotype of these animals. The mode of inheritance of the "double-muscling" conformation in the Kinsella DM population will also be examined.

C. Materials and Methods

The data for this study came from records on a DM population maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta. Some of the animals in Table I.1 and all of the animals in Table I.2 have been scored on a subjective scale of 1 (normal muscling) to 5 (extreme muscling), based on the degree of phenotypic expression of the "double-muscling" trait. This DM population included animals which either express the "double-muscling" conformation or were offspring of animals which expressed the trait or had produced offspring of a double-muscled type. Thus, within this population, animals vary from phenotypically normal to extreme in muscling.

D. Results and Discussion

In the breeding herds maintained at Kinsella, animals which were overtly heavily muscled would infrequently appear. In an effort to supply animals for studies designed to provide information on the growth and development of muscles in these animal types, a heavily (double) muscled population was initiated in 1967.

Origin of the "double-muscling" trait

A double-muscled Angus bull (8013, Table I.1), obtained from the Canada Department of Agriculture, was the initial sire used to introduce the "double-muscling" trait. Normal-muscled Angus, Charolais and Galloway cross-bred cows (Tables I.1 and I.3) were bred to this bull during the 1967 and 1968 breeding seasons. The resulting offspring were allocated to the DM population. In 1969, another source of "double-muscling" was introduced into the DM population by a Charolais double-muscled bull (7455, Table I.1). Normal-muscled cross-bred cows, predominately of Angus, Charolais, Galloway and Hereford breeds (Tables I.1 and I.3), were bred to this bull. The use of this Charolais sire in the DM population is clearly demonstrated in Table I.3 by the sharp increase in Charolais in the breed percentages of the calves born in 1970. In 1971, an overtly heavily muscled bull (3561) appeared in the Beef Synthetic (SY) population, and was used as one of the breeding bulls for the DM herd during the 1972 breeding season (Table I.1). The breed composition of this animal was predominately Angus, Charolais, Galloway and Brown Swiss. The breed percentages of the Kinsella SY herd have been reported elsewhere (Berg 1978). In 1972, a three-quarters Limousin and one-quarter Hereford double-muscled bull (5992) was purchased (Table I.1) and used during the 1973 and 1974 breeding seasons (Table I.1 and I.2), resulting in a sharp increase in Limousin and Hereford in the breed percentages of DM calves born during 1974 and 1975 (Table I.3). Subsequently, extreme-muscled DM breeding bulls were chosen from within the DM herd (Table I.2). Preceeding the purchase of cow 5193 and an extreme-muscled female calf 5196 (Table I.2) and their introduction into the DM herd in 1976, breeding cows were also exclusively chosen from within the DM population. Thus ,since 1976, the DM herd has been bred as a closed breeding population. However, the DM herd is open to extreme-muscled animals which may arise in other Kinsella populations.

Breed percentages of the DM population

The breed percentages of all calves born to the DM population for each year from 1967 to 1981 are shown in Table I.3. The introduction of the various breeds into the DM population was discussed in the previous section. The breed percentages of calves born to the DM population have begun to stabilize with little change occurring since 1978 (Table I.3). In 1981, the average breed percentages of the calves born to the DM population were Angus 51%, Galloway 14%, Hereford 13%, Charolais 12% and Limousin 8%, with minor contributions from other founding breeds (Table I.3). Of the breeding populations at Kinsella, the SY herd shows the greatest similarity in muscling- and fattening-type and in breed composition to the DM population.

Inheritance of the "double-muscling" conformation

The Double Muscled Syndrome or "double-muscling" has been recognized as a genetic disorder in cattle for nearly 200 years (Cully 1807, as cited by Oliver and Cartwright 1968). Despite the knowledge of its long existence in cattle populations, much is uncertain about the mode of inheritance of this syndrome. This uncertainty is due primarily to the difficulty involved in carrier or heterozygote identification because of the extreme variation in phenotypic expression of the "double-muscling" conformation. Carriers of this syndrome can either approach the phenotype of normal animals, double-muscled animals or be intermediate

(Oliver and Cartwright 1968; West 1974). Further, the amount of the "double-muscling" conformation expressed by the carrier may vary widely from one animal to the next, depending upon such factors as breed, age, sex and physical condition (Oliver and Cartwright 1968; Rollins *et al.* 1972; Kieffer and Cartwright 1980).

Various hypotheses concerning the mode of inheritance of the syndrome have been proposed. Most agree that the "double-muscling" conformation is mediated by a single, autosomal, gene pair (Kidwell *et al.* 1952; Oliver and Cartwright 1968; Kieffer *et al.* 1972a, b; Rollins *et al.* 1972; Kieffer and Cartwright 1980). Diallele (Sopena and Blanco in 1970) and triallele (Kronacher in 1934) models have also been proposed (as cited by Rollins *et al.* 1972). Disagreement arises in the single gene pair hypothesis as to whether the gene for "double-muscling" is partially dominant or incompletely recessive (Kidwell *et al.* 1952; Oliver and Cartwright 1968; Kieffer *et al.* 1972a, b; Rollins *et al.* 1972). This disagreement stems from the wide variation in phenotypic expression of the character in the heterozygote. Evidence gathered recently indicates that the "double-muscling" gene is incompletely recessive to the gene for normal-muscling (Kieffer and Cartwright 1980). It is very doubtful that there is a gene for normal muscling or a gene for "double-muscling". Rather there is a gene-complex resulting in many gene-products which dictate the phenotypic expression of muscling. Alteration in one specific gene may result in an abnormal gene-product which may alter the phenotypic expression of normal-muscling.

Although it is quite clear that the "double-muscling" gene does not conform to the criteria of classical Mendelian genetics, controlled mating studies carried out by Kieffer and Cartwright (1980) have shown that from the standpoint of "practical transmission genetics", the "double-muscling" gene follows the rules of recessive Mendelian inheritance. In these studies and in those by the present author, carriers were defined in the "classical sense", that is being synonymous with heterozygote and were determined on the basis of the animal's phenotype and breeding history. The one gene model for the inheritance of the Double Muscled Syndrome is assumed.

In studies in which double-muscled bulls or cows have been mated to double-muscled cows or bulls, the offspring were almost exclusively double-muscled (Oliver and Cartwright 1968; Kieffer *et al*. 1972a, b; Rollins *et al*. 1972; Kieffer and Cartwright 1980). The same researchers showed that double-muscled by normal matings produced carriers which varied in their phenotypic expression of the "double-muscling" conformation. Further, carriers mated to carriers produced double-muscled, carriers and normal calves in the ratio of 1:2:1 and carriers mated to double-muscled animals produced double-muscled and carrier calves in the ratio of 1:1 (Oliver and Cartwright 1968; Kieffer *et al*. 1972a, b; Rollins *et al*. 1972; Kieffer and Cartwright 1980).

Discrepancies between expected and observed results have been suggested to be due to mistaking carriers as double-muscled animals and/or normal animals as carriers (Oliver and Cartwright 1968; Kieffer and Cartwright 1980).

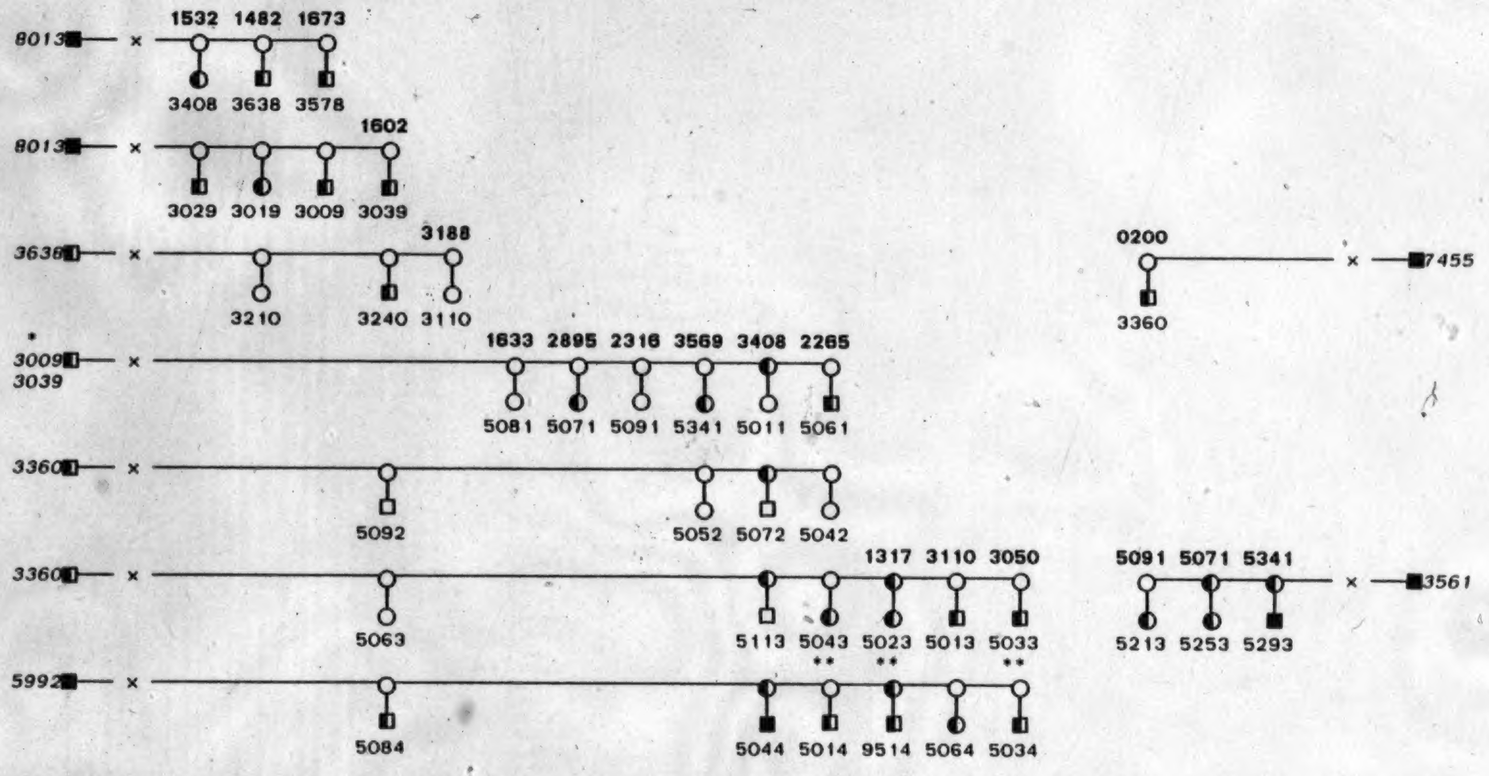
In the DM population maintained at Kinsella from 1967 to 1981, 62 fertile matings between double-muscled bulls and suspected carrier cows were made (Tables I.1 and I.2). These matings resulted in 29 double-muscled and 34 carrier calves which gave a ratio of double-muscled to carrier calves of 1:1.17. The carriers varied in phenotypic expression of the "double-muscling" conformation from being indistinguishable from normal animals to being extreme in muscling (for example, animals #5046 and #5027). Double-muscled bulls mated to normal cows only produced carrier calves which were variable in phenotypic expression of the "double-muscling" conformation (Tables I.1 and I.2). Only four carrier by carrier matings are shown in Tables I.1 and I.2. These matings only resulted in carriers and normal offspring. However, this number of matings is too small to accurately determine a ratio. Of 15 carrier by normal matings shown in Table I.1, seven carrier and eight normal animals were observed. The genotypic status of some of these animals is much less certain than that for the double-muscled x carrier matings because of their limited existence in the DM population. The observed result is in agreement with the expected result for recessive Mendelian inheritance. Double-muscled by double-muscled matings resulted in only four double-muscled calves (Table I.2). Determination of the genotype of each animal (Tables I.1 and I.2) was based on the breeding history and/or the phenotypic expression of the "double-muscling" conformation of each animal.

Of the 33 double-muscled calves born to the Kinsella DM population, 23 were male and 10 were female (Tables I.1 and I.2) giving a male to female ratio of 2.30:1. That of nondouble-muscled calves produced in the same herd approximated a male to female ratio of 1:1 (Tables I.1 and I.2). In previous studies by Rollins and others (Rollins *et al.* 1972) an overall male to female ratio of 3:1 in double-muscled animals was observed while that in nondouble-muscled animals was not significantly different from the 1:1 sex ratio. Thus, it is clear that the 1:1 sex ratio does not exist among double-muscled animals. Two alternative explanations have been proposed by Rollins *et al.* (1972). Firstly, there is a greater prenatal selection against double-muscled females than against double-muscled males. Alternatively, there are different modes of inheritance of the "double-muscling" trait between sexes. This latter alternative presupposes influence to the "double-muscling" trait by both the autosomal gene(s) and the sex chromosome. Experimental evidence gathered by Rollins *et al.* (1972) supports the former alternative. In addition, it may also be proposed that there are different degrees of expression of the "double-muscling" trait between sexes. An important aspect of this proposal may be the prenatal influence of the sex hormones.

The observations presented in Tables I.1 and I.2 are in agreement with those presented by Oliver and Cartwright (1968), Kieffer *et al.* (1972a, b), Rollins *et al.* (1972) and Kieffer and Cartwright (1980). It may, therefore, be suggested that the "double-muscling" gene behaves as a normal segregating recessive. However, this does not explain the extreme variation observed in the phenotypic expression of the "double-muscling" conformation in the heterozygote. Oliver and Cartwright (1968) suggested that this variation was caused by various alleles at other loci modifying the gene for the "double-muscling" conformation. In consideration of the present knowledge of genetics, one can re-state Oliver and Cartwright's hypothesis and say that this variation in the carrier may be due to the modifying effect of one or more gene-products on the phenotypic expression of the "double-muscling" conformation. Genes do not modify other genes directly. Thus, the phenotypic expression of "double-muscling" may depend on what proportion of the modifying genotype is inherited from each parent. In matings (for example, DM x carrier, carrier x carrier and carrier x normal) where there may be a potential for both the inheritance of genes which produce products that modify the phenotype towards normal-muscling and towards "double-muscling", variation in the phenotypic expression of the "double-muscling" conformation may be expected. In the case of double-muscled by double-muscled matings, genes which produce products that modify the "double-muscling" conformation towards normal-muscling may not be present or effective. This may be true when the "double-muscling" trait first arises in any given cattle population. However, in the Piedmont breed, years of selection for a more fit and productive double-muscled animal has resulted in an animal which is "double-muscled" without the associated reproductive problems normally associated with "double-muscling" in other breeds of cattle (Raimondi 1962). Breeding studies with the Piedmont breed suggest that genes have accumulated under the pressure of artifical and/or natural selection which produce products that modify the associated reproductive problems as well as tending to endow the "double-muscling" conformation with dominance (Raimondi 1962). Thus, the evidence presented here and by others (Oliver and Cartwright 1968; Kieffer *et al.* 1972a, b; Rollins *et al.* 1972; Kieffer and Cartwright 1980) suggests that the "double-muscling" conformation is mediated by a single recessive gene pair with variable expression in the heterozygote mediated by modifying gene-products. Selection of a more fit double-muscled animal may result in the accumulation of genes and thus gene-products which promote the trait to dominance (Fisher 1928, 1929).

Rollins *et al.* (1972) and Kieffer and Cartwright (1980) have described the mode of inheritance of the "double-muscling" trait and have made the assumption that the trait is controlled by a single gene pair. Genotypic categorization of double-muscled animals is based, to some degree, on this assumption. It is therefore not surprising that the data fit the model. The same conclusion could be made even if the expression of "double-muscling" behaved as a quantitative trait under the control of many genes. A quantitative trait becomes qualitative by classifying in discrete and few categories. Thus, the mode of inheritance of the "double-muscling" conformation still requires further clarification. Understanding the mode of inheritance of the Double Muscled Syndrome is important for the use of the "double-muscling" conformation for beef production (West *et al.* 1973; West 1974; Carroll *et al.* 1978; Kieffer and Cartwright 1980).

Table I.1 The pedigree tree and inheritance of the "double-muscling" trait for the "Double-Muscled" (DM) population maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta from 1967 to 1974.[1]



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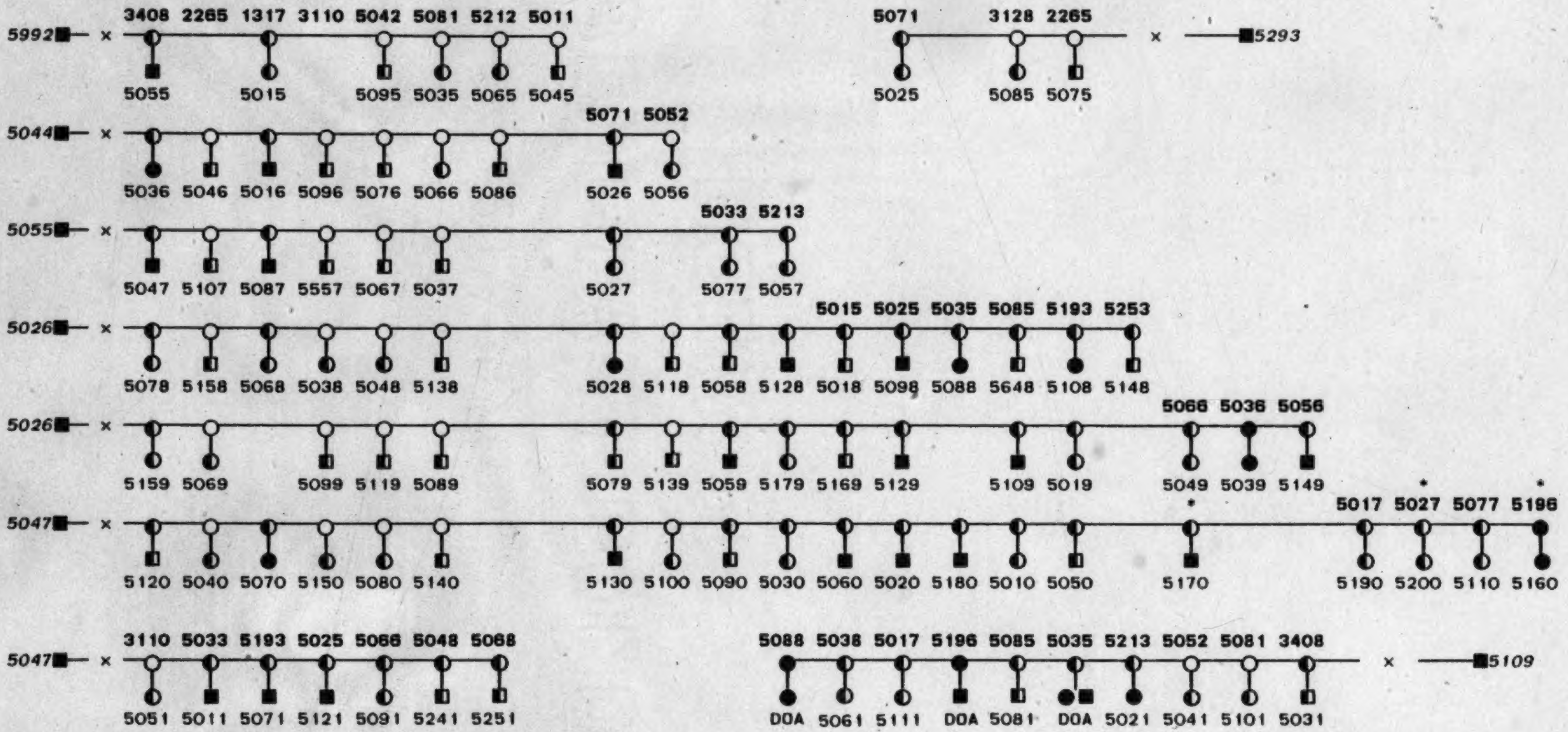
[1] The animal identification is represented by a sequence of four numbers. The last number in this sequence gives the year in which the animal was born. Dams which have the same last number as their offspring are 10 years apart in age (i.e., 160-62 and 509-72). Offspring of each dam occur in the same column. The sires are represented by *italic* numbers, the dams by **bold** numbers, and their offspring by normal printed numbers. Normal males and females are represented by □ and ○, respectively. More or less normal-muscled male and female carriers of the "double-muscling" trait are represented by ◧ and ◐, respectively, and overtly affected DM male and female animals by ■ and ●, respectively. The determination of the genotype of each animal was based on a subjective score for muscling and on the complete breeding history of each animal.

* Bulls 3009 and 3039 were placed with the same group of cows under range conditions. Exact cow bred by each bull is therefore unknown. Breed composition of both bulls is the same.

** These dams were bred by DM bull 3561.

Table I.2 The pedigree tree and inheritance of the "double-muscling" trait for the "Double-Muscled" (DM) population maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta from 1974 to 1981.[1]



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[1] The animal identification is represented by a sequence of four numbers. The last number in this sequence gives the year in which the animal was born. Dams which have the same last number as their offspring are 10 years apart in age (i.e., 340-68 and 507-78). The sires are represented by *italic* numbers, the dams by **bold** numbers, and their offspring by normal printed numbers. Normal males and females are represented by □ and ○, respectively. More or less normal-muscled male and female carriers of the "double-muscling" trait are represented by ◧ and ◐, respectively, and overtly affected DM male and female animals by ■ and ●, respectively. Offspring of each dam occur in the same column. The determination of the genotype of each animal was based on a subjective score for muscling and/or on the complete breeding history of each animal.

* These dams were bred by DM bull 5026. Calves designated DOA were dead at birth.

Table I.3 Average breed percentages of all calves born to the "Double-Muscled" (DM) population at Kinsella.

Breed	1967	1968	1969	1970	1971	1972	1973	1974	1975	1976	1977	1978	1979	1980	1981
Angus	100	75.00	79.17	52.05	51.95	43.44	50.56	33.91	32.21	40.28	39.46	44.06	42.99	45.95	50.73
Charolais		7.69	1.39	14.00	6.07	27.66	24.77	9.15	12.21	6.35	14.51	11.51	15.15	12.07	12.20
Galloway		17.31	19.44	23.44	18.62	12.19	14.81	15.31	12.56	15.10	14.84	14.32	13.88	16.26	14.32
Hereford				9.61	22.24	16.64	6.79	16.57	21.55	19.45	14.47	17.72	16.50	14.38	12.79
Brown Swiss				0.78	1.04	0.00	3.00	2.50	1.20	0.00	1.04	0.87	1.21	0.69	1.46
Brahman				0.13	0.07	0.08	0.04	0.06	0.08	0.07	0.06	0.07	0.06	0.06	0.06
Limousin								22.50	20.19	18.75	15.63	11.46	10.23	10.59	8.44
Animals[1]	1	13	9	32	24	10	25	10	13	9	12	18	22	27	15

[1] Number of animals contributing to the breed percentages.

II. ERYTHROCYTE FRAGILITY IN DOUBLE-MUSCLED CATTLE, CARRIERS AND NORMAL CATTLE[1]

A. Abstract

Osmotic fragility of erythrocytes was determined on heparinized whole blood, sampled from a group of 95 cattle consisting of 73 normal animals from three breed groups and 22 animals showing varying degrees of the "double-muscling" trait. Four age groups and two sex groups were sampled from each breed group. Mean corpuscular fragility (MCF) values (the NaCl equivalence producing 50% hemolysis) were interpolated from a fragility curve derived for each animal. The "Double-Muscled" (DM) breed group had significantly increased erythrocyte fragility as compared to the other breed groups. As animal age increased, the fragility of the erythrocytes was significantly decreased. Sex did not appear to influence erythrocyte fragility. Erythrocyte fragility was also related to the degree of phenotypic expression of the "double-muscling" trait. Phenotypically extreme-muscled DM cattle had increased erythrocyte fragility as compared to phenotypically normal- to moderate-muscled animals of the DM breed group. Erythrocyte fragility in two of the other breed groups overlapped into the erythrocyte fragility range of the phenotypically normal- to moderate-muscled DM animals,

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[1] A slightly modified version of this chapter has been published. Basarab, J.A., Berg, R.T. and Thompson, J.R. 1980. Erythrocyte fragility in "double-muscled" cattle. Can. J. Anim. Sci. 60: 869-876.

while the remaining breed group showed a decreased erythrocyte fragility. This overlapping provides adequate grounds for the rejection of the erythrocyte osmotic fragility test used in this study as a means of unequivocal identification of carriers of "double-muscling" in cattle. Despite the rejection of this test as a means of carrier detection, the suggestion (King *et al.* 1976a) that double-muscled cattle may have a "generalized membrane defect" is still considered valid.

B. Introduction

There are a number of reports on the physical, physiological, histological, histochemical and biochemical characteristics associated with the Double Muscled Syndrome (DMS) in cattle (Oliver and Cartwright 1968; Holmes and Ashmore 1972; Holmes *et al.* 1972a, b, 1973; Hendricks *et al.* 1973; Ashmore *et al.* 1974). The mode of inheritance of DMS or double-muscling in cattle is described as involving a single recessive gene pair with incomplete penetrance showing variable phenotypic expression in the heterozygote making detection of carriers quite difficult (Oliver and Cartwright 1968; Kieffer *et al.* 1972a, b; Rollins *et al.* 1972; Kieffer and Cartwright 1980; Chapter I).

King *et al.* (1976a) stated that the erythrocyte osmotic fragility test described in their report was a clearcut method for the detection of double-muscled carriers in cattle. Their approach was based on evidence showing that a "generalized membrane defect" may be one of the most consistent features of DMS in cattle (King and Basrur 1974; King 1975). Chemical analysis of muscle tissue from double-muscled cattle had previously shown that the syndrome is associated with an increased potassium/sodium ratio (Lawrie *et al.* 1964). Double-muscled cattle have also been shown to have elevated resting levels of plasma creatine phosphokinase (CPK) (Holmes *et al.* 1972c) and increased plasma and serum levels of CPK, lactate dehydrogenase, glutamate-oxaloacetate transaminase and potassium when stressed (Ashmore and Robinson 1969; Holmes *et al.* 1972b, c, 1973). Holmes and associates (Holmes *et al.* 1972b, c) have stated that during stress, CPK can be lost from muscles which show no gross or microscopic signs of necrosis. The above observations can be interpreted as evidence for the hypothesis (King *et al.* 1976a) of an alteration in the functional state of the skeletal muscle membranes in double-muscled cattle.

In order to control and utilize the "double-muscling" trait, additional investigation is required to further the knowledge of its inheritance and manifestations. The objectives of the present study were to examine the relationships of "double-muscling" and erythrocyte osmotic fragility and to examine the validity of the test as a detection method for carriers of the trait in cattle.

C. Materials and Methods

Ninety-five cattle, maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta, were used in this study. This group consisted of 73 animals from three breed groups showing normal muscling and 22 animals from a "Double-Muscled" (DM) breed group showing varying degrees of the "double-muscling" trait. With the exception of some of the original females, all animals within the DM population either express the "double-muscling" trait or are offspring of animals which either express the trait or had produced offspring of a double-muscled type (Chapter I). There were four age groups and two sexes per breed group (Table II.1). The four breed groups were DM, Beef Synthetic (SY), Dairy Synthetic (DY) and Hereford (HE) (Berg, 1978). Their average breed percentages are summarized in Table II.2. The HE, SY and DY breed groups were early-, intermediate- and late-fattening types (Price *et al.* 1977), respectively, and were included for comparison with the DM breed group. The SY breed group showed some similarity in breed composition (Table II.2) and in muscling and fattening type to the DM breed group.

Based on the degree of phenotypic expression of the "double-muscling" trait, the animals of the DM breed group were scored on a subjective scale of 1 (normal-muscling) to 5 (extreme-muscling). Nine of the 22 DM animals were categorized as extreme-muscled and 13 as phenotypically normal to moderate. Animals within the DM breed group categorized as phenotypically normal- to moderate-muscled were considered to be carriers of "double-muscling" if they had either produced double-muscled calves or were sired by an extreme DM bull in the DM population (Chapter I).

Blood sampling and analyses

Each animal was restrained in a cattle squeeze and 10 mL of venous blood were drawn into a heparinized Vacutainer. The sample was inverted several times and then immediately analyzed for erythrocyte osmotic fragility by exposing the whole blood to decreasing concentrations of buffered NaCl solution. The degree of hemolysis at each NaCl concentration was measured colorimetrically by the autoanalyzer method described by Gowdy and Koneman (1967). Nineteen buffered NaCl solutions, ranging in NaCl equivalence from 0.90 to 0.10%, were used. These solutions were prepared by the procedure described by Dacie and Lewis (1968). An erythrocyte osmotic fragility curve was calculated for each animal by plotting percent hemolysis against percent concentration NaCl solution.

Mean corpuscular fragility (MCF) values (the NaCl equivalence producing 50% hemolysis) were interpolated from each animal's fragility curve. These values were tested for significance by least squares analysis of variance for unequal numbers (Harvey 1960). Sources of variance were breed group (n=4), phenotype/breed group, sex (n=2), breed group x sex, age (n=4), breed group x age and sex x age. Those sources of variance with significant F-values were subjected to Student-Newman-Keuls multiple comparison of means (Steel and Torrie 1980).

D. Results and Discussion

The mean erythrocyte osmotic fragility curves for breed groups are shown in Fig. II.1. Erythrocytes from the DM breed group were found to be more susceptible to osmotic lysis than erythrocytes from the SY, DY and HE breed groups. The SY and DY breed groups demonstrated an increased erythrocyte osmotic fragility as compared to the HE breed group. The DY breed group gave an erythrocyte osmotic fragility very similar to that of the SY breed group. The least squares mean MCF values shown in Table II.3 demonstrate that the differences in erythrocyte osmotic fragility observed between the DM and other breed groups, between the SY and HE breed groups, and between the DY and HE breed groups were significant at the probability level of $P<0.05$. Comparison of the least squares mean MCF values for the SY and DY breed groups show that the erythrocyte osmotic fragility observed for the SY breed group was increased, though not significantly ($P>0.05$), from that of the DY breed group. Cattle breed group differences in erythrocyte osmotic fragility have been observed by other researchers (Evans and Turner 1965). The exact mechanism(s) of action accounting for this breed group difference in erythrocyte osmotic fragility is (are) unknown. Cooper (1969), Kuiper *et al.* (1971) and Sagawa and Shiraki (1978) have shown that differences in enzymes associated with erythrocytes, erythrocyte membrane lipid composition and blood components all influence the osmotic fragility of erythrocytes.

In this study, sex was not found to influence (P>0.05) bovine erythrocyte osmotic fragility. The least squares mean MCF values for all female and male animals were 0.493±0.003 and 0.494±0.005, respectively. This non-significant influence of sex on erythrocyte osmotic fragility has also been observed in other species (Jain 1973).

The mean erythrocyte osmotic fragility curves by age groups (Fig. II.2) demonstrate that the susceptibility of erythrocytes to osmotic lysis decreased considerably between the 7 to 8- and 43 to 79-mo age groups. Comparison of the least squares mean MCF values presented in Table II.3 show that the decline in erythrocyte osmotic fragility between the 7 to 8- and 43 to 79-mo age groups was significant at P<0.05. However, this decline in susceptibility as animal age increased did not behave in a completely linear fashion. For example, a non-significant (p>0.05) decrease in erythrocyte osmotic fragility was observed between the 7 to 8- and 19 to 20-mo age groups followed by a large significant decrease (P<0.05) in the erythrocyte osmotic fragility for the 31 to 32-mo age group. The reason for the sudden decrease in erythrocyte osmotic fragility between the 19 to 20- and 31 to 32-mo age groups, or the reason why erythrocyte osmotic fragility decreased as animal age increased, remains unknown. However, Grinna (1977) has cited evidence to show that in rat skeletal muscle and human red blood cells, membrane cholesterol, phospholipids and saturated fatty acid concentrations increase during animal aging. Increased levels of these red cell membrane components in humans have been shown to result in decreased erythrocyte osmotic fragility and altered membrane fluidity and permeability (Cooper 1969). Certainly other changes do occur during animal aging, for example hormonal and enzymatic changes, but their interaction with plasma membranes are not well understood (Grinna 1977).

No significant breed group x sex, breed group x age or sex x age interactions were observed for the data in this study.

The least squares mean MCF values for the extreme-muscled and phenotypically normal- to moderate-muscled animals from the DM population and for the SY, DY and HE breed groups are given in Table II.4. The extreme-muscled DM animals demonstrated an increased ($P<0.05$) susceptibility of their erythrocytes to osmotic lysis as compared to the phenotypically normal- to moderate-muscled animals in the DM group and to the SY, DY and HE breed groups. Animals within the DM breed group categorized as phenotypically normal- to moderate-muscled carriers of the "double-muscling" trait did not differ significantly ($P>0.05$) in their erythrocyte osmotic fragility from the SY or the DY breed groups. The phenotypically normal- to moderate-muscled DM animals did demonstrate a significantly ($P<0.05$) increased erythrocyte osmotic fragility compared to the HE breed group. These findings are, in part, similar to those presented by King *et al.* (1976a). Their results showed a definite distinction between the erythrocyte osmotic fragility of the extreme-muscled DM animals and the "more or less normal-muscled carriers" of the "double-muscling" trait ($P<0.05$). However, the data presented by King *et al.* (1976a) also showed that both extreme-muscled DM animals and the "more or less normal-muscled carriers of the trait" had an increased ($P<0.05$) susceptibility of their erythrocytes to osmotic lysis, as compared to normal Charolais cattle. The animals used by King and associates (King 1975; King *et al.* 1976a) were from the same DM population as those used in the present study. Since all breeding cows in this DM population were bred to affected DM sires (Chapter I), the categorization of animals in this population as normal on the criterion, according to King (1975), that they were "neither grossly affected nor siblings or parents of affected animals" is incorrect. In order for this categorization to be correct for the "Kinsella" DM population, animals outside this population would have to be used. As shown in this chapter, breed group and animal age influence erythrocyte osmotic fragility, hence, selection of an appropriate normal control breed group and correction for animal age are important when examining bovine erythrocyte osmotic fragility.

The overlapping of erythrocyte osmotic fragility observed for the phenotypically normal- to moderate-muscled DM animals, which would include any normal-muscled carriers of the major DM gene, has also been observed for many of the other characteristics associated with DMS (Oliver and Cartwright 1968; Holmes and Ashmore 1972; Holmes *et al.* 1972a, b, 1973; Hendricks *et al.* 1973; Ashmore *et al.* 1974; West 1974). A possible explanation for this overlapping observed for the phenotypically normal- to moderate-muscled DM animals into the erythrocyte fragility range of animals in the other breed groups is that erythrocyte fragility may be related to the degree of phenotypic expression of "double-muscling" and thus "normal" carriers would be similar in expression of erythrocyte fragility to normal non-carriers. Thus, the erythrocyte osmotic fragility test used in the present study cannot be used to unequivocally identify the carriers of "double-muscling" in cattle.

The increase in erthyrocyte osmotic fragility observed for extreme-muscled DM cattle in this study and by King and associates (King 1975; King *et al.* 1976a) is suggestive of membrane structural alterations in erythrocytes. It is conceivable that the membrane fragility seen in erythrocytes extends to membranes in other tissues and therefore may be indicative of a generalized membrane defect in double-muscled cattle. The elevated levels of plasma lactate dehydrogenase (King 1975), the increased muscle potassium/sodium ratio (Lawrie *et al.* 1964), as well as the degeneration and disruption of mitochondria, transverse tubular system and sarcolemma observed in skeletal muscles from extreme-muscled DM animals (King and Basrur 1974; King 1975) also supports this hypothesis. Other degenerative changes observed in the skeletal muscles from extreme-muscled DM cattle and to a lesser extent in carriers were disorientation of the cross striations, disruption and degeneration of the contractile elements, fragmentation of the myofibils, degeneration of neuromuscular junctions and accumulation of glycogen and lysosomes (King 1975). King (1975) stated that these changes are common to human neuromuscular myopathies. These observations taken together with studies showing that alien innervation of myofibers can alter muscle metabolism and hence muscle fiber type (Buller *et al.* 1960; Guth 1968; Guth *et al.* 1968; Mair and Tome 1972; Fardeau 1973; Dubowitz 1973; Swatland 1974) and the observation of altered innervation of myofibers in extreme-muscled DM cattle (Swatland 1973), prompted King (1975) to suggest that the hypertrophy and hyperplasia of muscles in double-muscled cattle results from altered neuromuscular contact at the membrane level, which in turn alters cellular metabolism. King (1975) also suggested that the structural alterations in the cell membranes in extreme-muscled and carrier animals led to this proposed disruption of neuromuscular contact. However, the greater capacity of muscles from double-muscled animals to produce lactic acid and the structural damage caused by its build up in muscles may confound King's hypothesis.

Thus, it may be suggested that the primary alteration caused by the gene or genes for "double-muscling" may be in either a structural or enzymatic component of the cell membrane or may reflect an alteration in cell metabolism, possibly resulting from an endocrine alteration in these animals. Further insight into the biochemical alteration responsible for DMS in cattle may assist in the development of an accurate detection method for carriers of this condition.

Table II.1 Number of animals tested for erythrocyte osmotic fragility classified by breed group, sex and age.

Breed group	Sex	Age (months)			
		7-8	19-20	31-32	43-79
DM	F	4	4	3	4
	M	6	1		
SY	F	4	4	4	4
	M	4	4	1	
DY	F	4	4	4	4
	M	4	1	1	
HE	F	4	4	4	4
	M	5	4	1	

Table II.2 Average breed percentages[1] in the breed groups

Breed	Breed group			
	DM	SY	DY	HE
Angus	39.3	34.0	16.3	
Charolais	13.9	35.7	0.6	
Galloway	14.7	21.9	8.4	
Hereford	19.1	3.0	4.3	100
Limousin	11.8			
Holstein			30.8	
Brown Swiss	1.1	5.2	29.7	
Others	0.1	0.2	9.9[2]	

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[1] Calculated from those animals which were used in this study.

[2] Includes Simmental and Guernsey.

Table II.3 Least squares mean MCF[1] values ± SEM by breed group and animal age.

Breed group	Animals[2]	MCF	SEM
DM	22	0.512*a*	0.006
SY	25	0.498*b*	0.005
DY	22	0.490*b*	0.006
HE	26	0.475*c*	0.005
Animal age (months)			
7-8	35	0.509*a*	0.004
19-20	26	0.503*a*	0.005
31-32	18	0.487*b*	0.007
43-79	16	0.477*b*	0.006
Error mean square		0.00042	

[1] Mean corpuscular fragility.

[2] Number of animals per breed or age group.

*abc*Means in the same column within breed group or age group with different italicized letters differ significantly at $P<0.05$.

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Table II.4 Least squares mean MCF[1] values ± SEM by DM phenotype and normal breed group.

Breed group	Phenotype[2]	Animals[3]	MCF	SEM
DM	Extreme-Muscled	9	0.525*a*	0.009
DM	Moderate to Normal	13	0.499*b*	0.009
SY	Normal	25	0.498*b*	0.005
DY	Normal	22	0.490*b*	0.006
HE	Normal	26	0.475*c*	0.005
Error mean square			0.00042	

[1] Mean corpuscular fragility.

[2] Based on a subjective score for muscling.

[3] Number of animals per group.

*abc*Means in the same column with different italicized letters differ significantly at $P<0.05$.

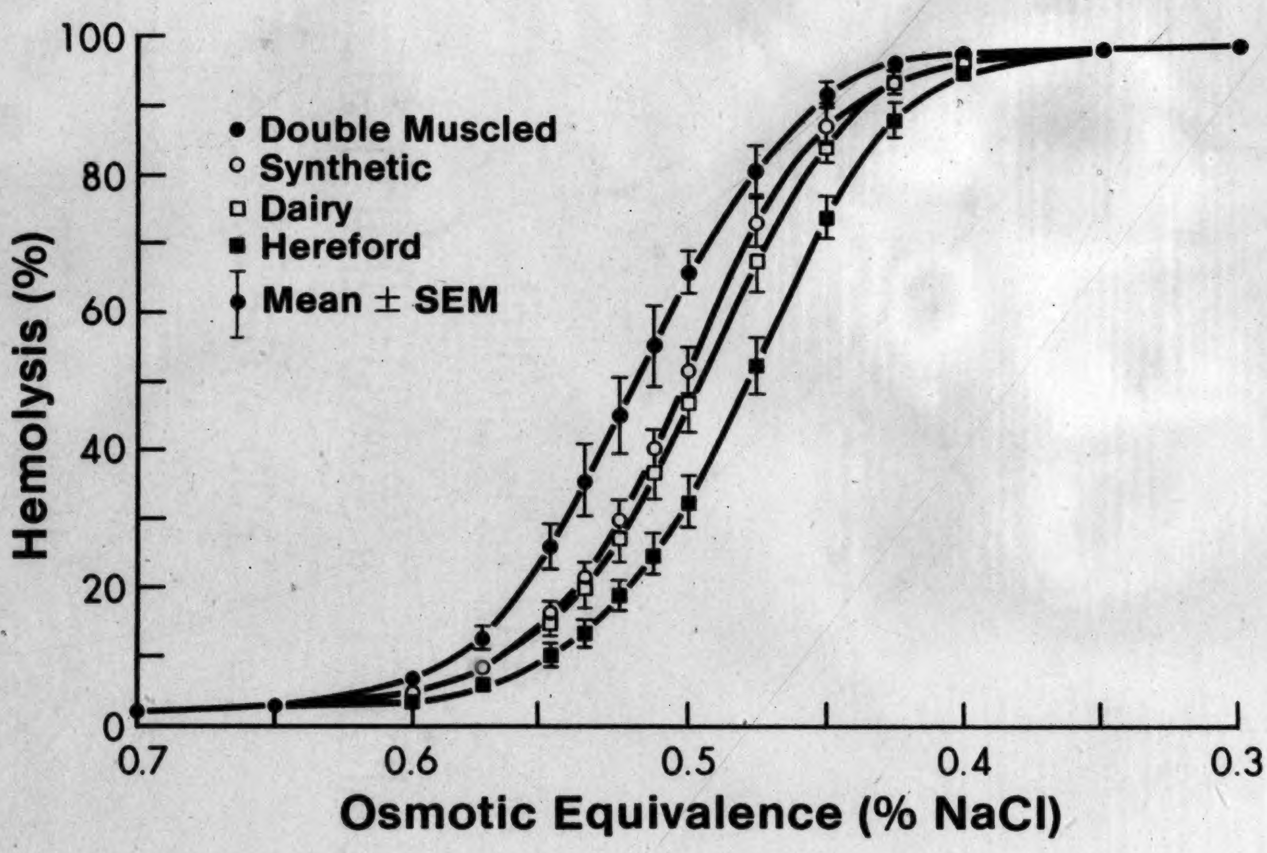

Figure II.1 Mean erythrocyte osmotic fragility curves for breed groups.

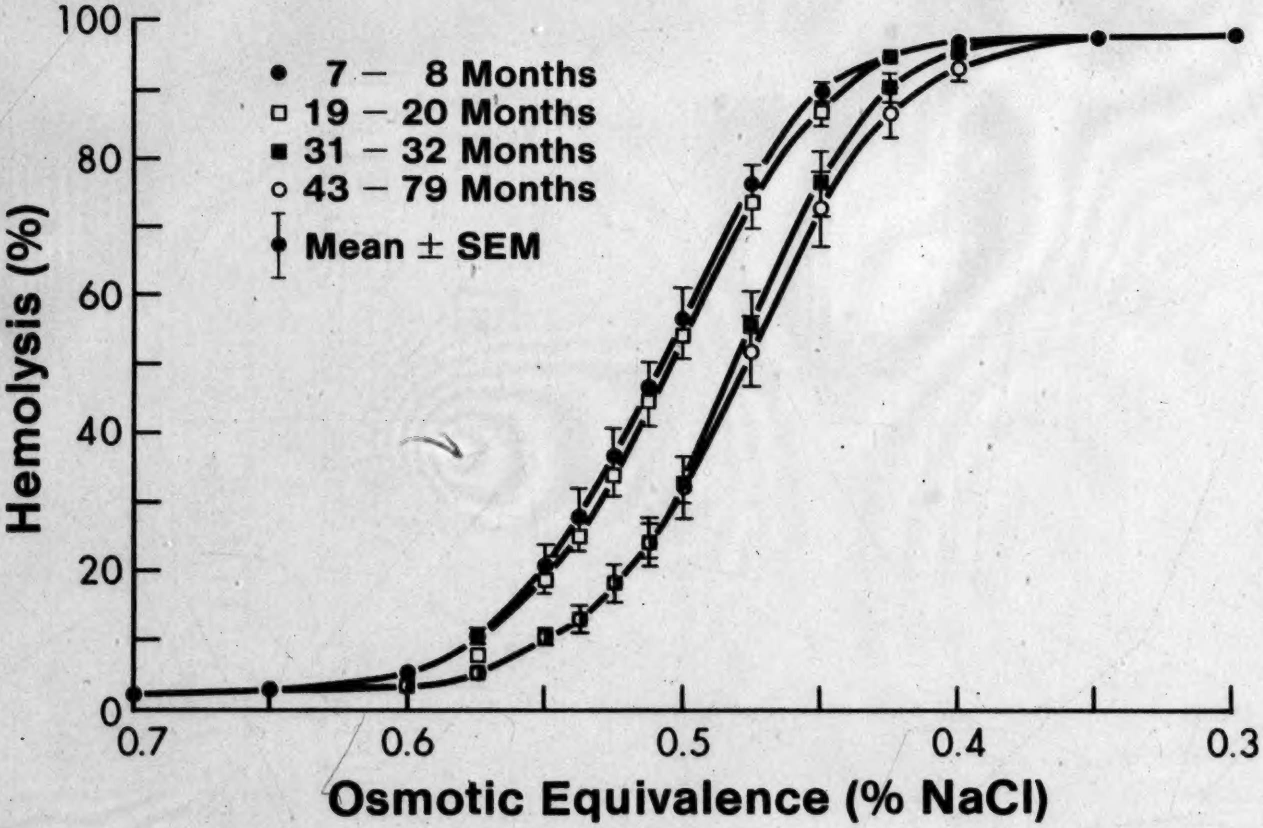

Figure II.2 Mean erythrocyte osmotic fragility curves for animal age groups.

III. THYROID HORMONE CONCENTRATIONS IN DOUBLE-MUSCLED CATTLE, CARRIERS AND NORMAL CATTLE

A. Abstract

Serum triiodothyronine (T_3) and thyroxine (T_4) concentrations were determined in 43 cattle of a Beef Synthetic (SY) breed group and 44 cattle of a "Double-Muscled" (DM) breed group. There were six age groups and two sexes per breed group. Animals within the DM breed group were categorized as phenotypically normal, moderate or extreme based on the degree of phenotypic expression of the "double-muscling" trait. Serum T_3 and T_4 concentrations tended to decrease as animal age increased. Sex did not significantly influence T_3 and T_4 concentrations. Serum T_3 concentration was significantly influenced by the degree of phenotypic expression of the "double-muscling" trait with phenotypically extreme-muscled DM animals demonstrating an increased T_3 concentration as compared to phenotypically normal DM and SY cattle. Serum T_3 concentrations in the phenotypically moderate-muscled DM cattle were intermediate. A non-significant trend was observed for the phenotypically extreme-muscled DM cattle to have elevated serum T_4 concentrations as compared to the other phenotypes. The phenotypically moderate-muscled DM and normal SY cattle tended to be intermediate to phenotypically extreme- and normal-muscled DM cattle in serum T_4 concentrations. The concentrations of circulating serum T_3 and T_4 observed in

the phenotypically extreme-muscled DM animals under the experimental conditions of this study may suggest a different response of the hypothalamo-hypophysial-thyroid axis to stress or an altered thyroid hormone metabolism in this animal type relative to normal cattle.

B. Introduction

The Double Muscled Syndrome (DMS) in cattle is an inherited abnormality of skeletal muscle growth and development characterized by increased muscularity, decreased fat deposits and increased susceptibility to stress (Oliver and Cartwright 1968; Holmes and Ashmore 1972; Holmes *et al.* 1972a, b, c, 1973; Ashmore *et al.* 1974; West 1974). The fundamental biochemical alteration responsible for the DMS in cattle is unknown. Several investigators (Pomeroy and Williams 1962; Vissac *et al.* 1974) have suggested that the primary defect may be due to an endocrine alteration. Others (Boccard and Dumont 1974) have speculated that the reduced collagen content in the enlarged muscles of DMS cattle may be the primary cause of the generalized muscular hypertrophy. King and associates (King 1975; King *et al.* 1976a) have presented evidence to suggest that the defect may be expressed at the cell membrane level.

A somewhat similar condition of muscular hypertrophy and stress susceptibility, of which the etiology has been more extensively studied, exists in stress susceptible (SS) swine (Ashmore 1974). The SS swine have been reported to have an increased erythrocyte osmotic fragility (Harrison and Verberg 1973) indicative of a generalized cell membrane defect (King *et al.* 1976b), increased serum thyroxine (T_4) concentrations (Eikelenboom and Weiss 1972) and metabolic clearance rates of triiodothyronine (T_3) and T_4 (Marple 1977; Marple *et al.* 1977) and an increased basal metabolic rate (Williams *et al.* 1977). Marple *et al.* (1977) suggest that the membrane defect, at least in the case of the sarcoplasmic reticulum in SS swine, is due to an over stimulation by thyroid hormones as it has been shown that thyroid hormones alter the lipid composition and hence fluidity and functionality of membrane systems (Hulbert *et al.* 1976). It is therefore conceivable that an altered thyroid hormone metabolism may be common to both DMS cattle and SS swine.

In the present study, serum T_3 and T_4 concentrations were measured as indicators of an altered thyroid status in double-muscled as compared to normal cattle.

C. Materials and Methods

The animals used in this study were from The University of Alberta beef breeding herds stationed at Kinsella, Alberta. They consisted of 43 cattle of a Beef Synthetic (SY) breed group and 44 cattle of a "Double Muscled" (DM) breed group (Basarab *et al.* 1980). There were six age groups and two sexes per breed group (Table III.1). Animals within the DM breed group were scored on a subjective scale of 1 (normal-muscling) to 5 (extreme-muscling) based on the degree of phenotypic expression of the "double-muscling" trait. Animals receiving a score of 1 to 2, 3 to 4 and 5 were categorized as phenotypically normal, moderate and extreme, respectively. In the present study, animals of the DM population categorized as phenotypically normal to moderate were considered carriers of the "double-muscling" conformation (Basarab *et al.* 1980; Chapter I). The SY breed group was included as a normal-muscled group which showed greatest similarity in breed composition and muscling- and fattening-type to the DM breed group (Basarab *et al.* 1980).

Blood sampling and analyses

Each animal was restrained in a cattle squeeze and 15 mL of jugular blood were drawn into a Vacutainer. After the blood samples had clotted at room temperature, serum was obtained by centrifugation and stored at -20°C. All animals were sampled within a period of two days. Serum concentrations of T_3 and T_4 were determined by radioimmunoassay (Chopra *et al.* 1972; Calbiochem 1975) and unbound label was removed with dextran coated charcoal.

Statistical analyses

Serum T_3 and T_4 concentrations were tested for significance by least squares analysis of variance (Harvey 1960). Due to the nature of the missing cells in the experimental design (Table III.1) the data were analyzed in three separate analyses: 1. 3 to 4-mo old males plus females across all phenotypes; 2. 3 to 4- plus 27 to 28-mo old females across all phenotypes and; 3. females of the moderate and normal phenotypes across all age groups. Sampling time interval (0700 to 1000-, 1000 to 1300-, 1300 to 1600- and 1600 to 1900-hours) and day were included as sources of variation for each analysis. Those sources of variation with significant F-values were subjected to Student-Newman-Kuels multiple comparision of means (Steel and Torrie 1980).The correlation (Steel and Torrie 1980) between serum T_3 and T_4 levels across all data was also determined.

D. Results

The effect of animal age on serum T_3 and T_4 concentrations is reported in Table III.2. Serum T_3 concentrations decreased as animal age increased, with significant ($P<0.05$) decreases occurring between the 3 to 4- and 15 to 16-mo age groups and between the 15 to 16- and 87 to 111-mo age groups. Serum T_4 concentrations were significantly ($P<0.05$) greater in the 15 to 16-mo age group than in all other age groups except for the 27 to 28-mo age group. The concentrations in all groups other than the 15 to 16-mo age group were similar.

Sex, day of sampling and time of sampling had no significant ($P>0.05$) influence on serum T_3 and T_4 concentrations. No significant ($P>0.05$) interaction effects were observed.

Least squares means by DM phenotype and SY breed group for both the combined 3 to 4-mo old male plus female calves and for the combined 3 to 4- plus 27 to 28-mo old female cattle for serum T_3 and T_4 concentrations are given in Table III.3. The serum T_3 concentration was significantly ($P<0.05$) greater in both phenotypically extreme-muscled DM groups when compared to either the phenotypically normal DM or SY cattle. Serum T_3 concentrations in the phenotypically moderate-muscled DM calves did not differ significantly ($P>0.05$) from the other phenotypes with a mean serum T_3 concentration intermediate to the phenotypically extreme- and normal-muscled DM calves. However, the serum T_3 concentration in the combined 3 to 4- plus 27 to 28-mo old phenotypically moderate-muscled DM female cattle was significantly ($P<0.05$) decreased as compared to the phenotypically extreme-muscled DM female cattle. The phenotypically moderate- and normal-muscled DM and normal SY female cattle did not have significantly ($P>0.05$) different serum T_3 concentrations, though the least squares mean T_3 concentration for the phenotypically moderate DM cattle was elevated over that for the phenotypically normal DM and normal SY female cattle. Mean serum T_4 concentrations by phenotype were variable and not significantly ($P>0.05$) different. However, a trend was observed for the T_4 concentration to be greater in the phenotypically extreme-muscled DM cattle ($P<0.1$ in the case of the 3 to 4-mo old male plus female calves) as compared to the other

DM phenotypes and the SY cattle, with phenotypically moderate DM and normal SY cattle being intermediate.

Least squares mean serum T_3 and T_4 concentrations in the moderate- and normal-muscled DM phenotypes and the SY breed group for females over all age groups are given in Table III.4. Across age groups the serum T_3 concentration for the phenotypically moderate DM female cattle was significantly ($P<0.05$) increased relative to the normal DM phenotype. The SY female cattle over all age groups did not differ significantly ($P>0.05$) in their serum T_3 concentrations from the phenotypically moderate and normal DM female animals. Serum T_4 concentrations by phenotype (Table III.4) were not significantly ($P>0.05$) different, though again least squares mean serum T_4 concentrations for the phenotypically moderate DM and the normal SY cattle were elevated over those for phenotypically normal DM cattle.

No significant ($P>0.05$) correlation was observed between T_3 and T_4 concentrations from the same serum sample ($r=0.20$).

E. Discussion

The serum T_3 and T_4 concentrations observed in the present study for normal-muscled carriers of "double-muscling" and normal SY cattle are within the range of those values reported by others for cattle of similar age (Kallfelz and Erali 1973; Kahl *et al.* 1977; Strath *et al.* 1980). Serum T_3, and to a lesser extent serum T_4

concentrations observed for extreme DM cattle tended to be higher than the literature values reported for normal cattle of similar ages.

Animal aging is known to affect plasma concentrations of T_3 and T_4 (Kallfelz and Evali 1973) as well as secretion rates and rates of metabolism of thyroid hormones (Hales *et al.* 1976). From the second day to the fourth or fifth month of life in calves, both plasma T_3 and T_4 concentrations gradually increase to 1.4 to 1.5 ng/mL and 50 to 60 ng/mL, respectively (Kahl *et al.* 1977). Thyroid secretion rate per unit body weight has been shown to increase during the first six to nine months of life in lambs (Falconer and Draper 1967). In adult sheep there is a gradual decrease in thyroid output with increasing age (Falconer and Draper 1967). Similar age related trends in plasma T_3 and T_4 concentrations have been observed in other mammalian species (Slebodzinski 1965; Fisher and Odell 1969; Nathanielsz 1969; Nathanielsz *et al.* 1973; Abuid *et al.* 1974; Erenberg *et al.* 1974; Dussault and Labrie 1975; Hales *et al.* 1976). The age related changes in serum T_3 and T_4 concentrations observed in the present study are similar to those reported in the literature.

Evidence has been presented by Larsen (1972) that elevated serum T_3 concentrations are a good indicator of thyroid hyperfunction. Thus, the increased serum T_3 level observed in extreme-muscled DM cattle indicate that this animal type has a more active thyroid relative to carriers of the "double-muscling" trait and to normal non-carrier animals. However, serum levels of thyroid hormones have not been conclusive in assessing thyroid status (Marple *et al.* 1977) and rates of hormone utilization or metabolism are a more effective criterion.

In the present study, serum T_3 and T_4 concentrations were determined from blood obtained by venipuncture. Emotional stresses induced by confinement, isolation, noise or discomfort have been shown to result in increased thyroid hormone secretion (Falconer and Hetzel 1964). Strath *et al.* (1980) have presented data which suggest that double-muscled cattle have an abnormal thyroid response to various stressors (for example, heat and nutrition). Holmes *et al.* (1972a) observed that the double-muscled genotypes of cattle were more temperamental. Therefore, it could be suggested that the trend toward a decreased serum T_3 and T_4 concentration in normal- compared to extreme-muscled DM cattle in the present study, may reflect their susceptibility to stress. In short, animals manifesting the "double-muscling" trait in cattle may have a lower threshold of sensitivity to stimulation than normal cattle. However, Strath *et al.* (1980) have also shown an elevated resting serum T_3 level in extreme DM cattle relative to carriers. This indicates that the elevated serum T_3 levels observed in extreme DM cattle in the present study, may be due to more than the inducement of handling stresses, possibly a hyperactive thyroid.

The intermediacy of serum T_3 and, to a lesser degree, serum T_4 concentrations observed for the phenotypically moderate DM cattle is characteristic of many other traits associated with DMS in cattle (Oliver and Cartwright 1968; Holmes and Ashmore 1972; Holmes *et al*. 1972a, b, 1973; Hendricks *et al*. 1973; Ashmore *et al*. 1974; West 1974). Since phenotypically normal and moderate DM cattle include any normal-muscled carriers of the major DM gene, it could be suggested that serum T_3 and T_4 concentrations are related to the degree of phenotypic expression of the "double-muscling" trait and thus would overlap into the serum T_3 and T_4 range of normal non carriers (e.g., SY breed group). However, the significantly ($P<0.05$) elevated serum T_3 and the trend to have elevated T_4 levels observed in the phenotypically extreme DM cattle does suggest that these animals exhibit a circulating thyroid hormone pattern different from normal cattle under the present experimental conditions. Whether this difference is due to an increased hypothalamic response to stress or due to an altered thyroid hormone metabolism remains obscure. Subsequent studies, carried out partly as a result of the present study, revealed that extreme-muscled DM cattle have an increased metabolic rate, T_3 pool size and T_3 irreversible loss relative to carriers of "double-muscling" (Strath *et al*. 1981), suggestive that this animal type is hyperthyroid.

Table III.1 Number of animals measured for serum triiodothyronine and thyroxine concentrations classified by breed group, phenotype, sex and age.

			Age (months)					
Breed group	Phenotype	Sex	3 to 4	15 to 16	27 to 28	39 to 40	51 to 75	87 to 111
DM	Extreme	F	1		3			
		M	1					
DM	Moderate	F	5	2	1		3	2
		M	5					
DM	Normal	F	1	6	1	4	2	4
		M	3					
SY	Normal	F	7	8	5	4	6	4
		M	9					

Table III.2 Least squares means ± SEM of serum triiodothyronine and thyroxine concentrations by age for females of the combined moderate and normal DM phenotypes and SY breed group.

	Age (months)						
	3-4	15-16	27-28	39-40	51-75	87-111	EMS[1]
Triiodothyronine (ng/mL)	2.94*a* ±0.15	1.86*b* ±0.15	1.53*bc* ±0.20	1.52*bc* ±0.20	1.41*bc* ±0.15	1.18*c* ±0.18	0.2336
Thyroxine (ng/mL)	44.7 *a* ±3.1	64.1 *b* ±3.0	55.4 *ab* ±4.1	42.4 *a* ±4.0	48.3 *a* ±3.1	45.9 *a* ±3.6	9.3893

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[1] Error mean squares.

*abc*Means in the same row with different italicized letters differ significantly at $P<0.05$.

Table III.3 Least squares means ± SEM of serum triiodothyronine and thyroxine concentrations for cattle by DM phenotype and normal SY breed group.

Breed group	Phenotype[1]	male and female calves[2]		female cattle[3]	
		Triiodothyronine (ng/mL)	Thyroxine (ng/mL)	Triiodothyronine (ng/mL)	Thyroxine (ng/mL)
DM	Extreme	4.09 ± 0.46*a*	64.1 ± 8.4	4.18 ± 0.38*a*	65.7 ± 5.8
DM	Moderate	3.23 ± 0.25*ab*	48.9 ± 4.6	2.46 ± 0.45*b*	47.2 ± 6.8
DM	Normal	2.32 ± 0.39*b*	43.5 ± 7.1	1.48 ± 0.67*b*	33.5 ± 10.2
SY	Normal	2.43 ± 0.32*b*	49.5 ± 5.9	2.42 ± 0.39*b*	58.6 ± 5.9
Error mean square		0.3470	11.7600	0.3321	7.6630

[1] Based on a subjective score for muscling.
[2] Combined 3-4 month old male and female calves.
[3] Combined 3-4 and 27-28 month old female cattle.
*ab*Means in the same column with different italicized letters differ significantly at $P<0.05$.

Table III.4 Least squares means ± SEM of serum triiodothyronine and thyroxine concentrations in the moderate and normal DM phenotypes and the normal SY breed group for females over all age groups.

Breed group	Phenotype'	Triiodothyronine (ng/mL)	Thyroxine (ng/mL)
DM	Moderate	2.07 ± 0.24*a*	50.8 ± 4.8
DM	Normal	1.38 ± 0.22*b*	44.8 ± 4.4
SY	Normal	1.77 ± 0.23*ab*	54.8 ± 4.6
Error mean square		0.2336	9.3890

' Based on a subjective score for muscling.

*ab*Means in the same column with different italicized letters differ significantly at P<0.05.

IV. THE LIPID COMPOSITION OF ERYTHROCYTE MEMBRANES FROM DOUBLE-MUSCLED CATTLE AND NORMAL-MUSCLED CARRIERS

A. Abstract

This study compared the lipid composition of erythrocyte membranes from 11 phenotypically extreme-muscled and 11 phenotypically normal-muscled cattle of a "Double-Muscled" (DM) breed group. There were two age groups and two sexes within each phenotype. The lipid class composition and the fatty acid patterns of the total phospholipid, sphingomyelin and phosphatidylethanolamine fractions in erythrocyte membranes were found to be significantly influenced by both sex and age of the animal. The distribution of cholesterol and of the different molecular species of phospholipids in erythrocyte membranes from phenotypically extreme- and normal-muscled DM cattle were similar. Extreme-muscled DM cattle demonstrated significant differences in the fatty acid composition of their erythrocyte membrane sphingomyelin and phosphatidylethanolamine fractions as compared to normal-muscled carriers of the "double-muscling" trait. The most notable differences were in the relative concentrations of 16:0 and 18:1 in sphingomyelin. The fatty acid composition of erythrocyte membrane sphingomyelin from extreme-muscled DM cattle demonstrated significantly increased relative concentrations of total saturated and total polyunsaturated fatty acids as well as a decreased

relative concentration of total monounsaturated fatty acids as compared to normal-muscled carriers of "double-muscling". A similar trend was observed in the proportions of total saturated and total monounsaturated fatty acids in the erythrocyte membrane phosphatidylethanolamine of extreme-muscled DM cattle. The phenotypic differences observed in the present study support the suggestion that double-muscled cattle may have structural alterations in their erythrocyte membranes. These findings may also be indicative of a generalized membrane alteration in cattle exhibiting the Double Muscled Syndrome.

B. Introduction

It has been suggested that the Double Muscled Syndrome in cattle is associated with a generalized membrane defect (King 1975; King *et al.* 1976a). Results obtained previously by Lawrie *et al.* (1964), demonstrating an increased intramuscular potassium:sodium ratio, can also be interpreted as evidence for an altered cell membrane in cattle exhibiting the Double Muscled Syndrome. The red blood cell, by virtue of its ease of preparation and isolation and lack of subcellular organelles is an excellent membrane for investigating this proposed membrane defect. King *et al.* (1976a) and Basarab *et al.* (1980) have demonstrated an increased erythrocyte osmotic fragility in phenotypically extreme DM cattle as compared to carriers of the syndrome (Chapter II). Erythrocyte osmotic fragility is closely associated with the lipid composition of the erythrocyte membrane (Cooper 1969; Kuiper *et al.* 1971; Nelson 1972; Van Deenen and De Gier 1974; Sagawa and Shiraki 1978). Thus, double-muscled cattle may exhibit alterations in the lipid composition of their erythrocyte membranes. The purpose of the present study was to examine the lipid composition of the erythrocyte membranes from phenotypically extreme-muscled and phenotypically normal-muscled cattle from a "Double-Muscled" (DM) population.

C. Materials and Methods

Twenty-two cattle from a DM breed group, maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta, were chosen for this study. Eleven of these animals were subjectively categorized as phenotypically extreme-muscled and 11 as phenotypically normal-muscled, based on the degree of phenotypic expression of the "double muscling" trait. Animals categorized as phenotypically normal-muscled were carriers of the "double-muscling" trait since they were offspring of animals which either express the trait or have produced offspring of a "double-muscled" type (Chapter I). There were two age and two sex groups within each of the phenotypically normal- and extreme-muscled groups.

Blood sampling and erythrocyte membrane preparation

Sixty mL of jugular blood were drawn from each animal by venipuncture equally dispersed into three heparinized

Vacutainers. Erythrocytes were immediately separated from plasma by centrifugation at 1000 x g for 10 min at 20°C. The plasma and part of the buffy coat were removed by careful aspiration. Erythrocytes were suspended in 310 imOsM (ideal milliosmolarity) isotonic tris buffer (0.172M, pH 7.6, 4°C) to an approximate hematocrit of 50%. The three erythrocyte suspensions from each animal were gently inverted several times, pooled and placed on ice. The procedures for washing the erythrocytes and preparation of erythrocyte membranes are described in detail by Hanahan and Ekholm (1974). The initial hemolyzing buffer (20 imOsM tris buffer, 5mM $MgCl_2.6H_2O$, pH 7.6, 4°C), used in this study, contained $MgCl_2$ which has been shown to prevent membrane fragmentation and loss of membrane lipid and protein in bovine erythrocytes (Hanahan and Ekholm 1974). Erythrocyte membrane preparation was carried out in triplicate per animal and was completed on the same day as the blood samples were collected.

Extraction and analyses of membrane lipid

The three erythrocyte membrane preparations per animal were transferred to a 50 mL screw-capped glass tube using a total of 2 mL distilled deionized water. The lipid portion of each preparation was twice extracted with 15 mL chloroform-methanol (2:1, v/v), with centrifugation at 1000 x g for 5 min at 20°C after each extraction. All solvents used in sample preparation and lipid separation were purified by distillation. The chloroform phases from each animal were pooled and evaporated to dryness under nitrogen. The erythrocyte membrane lipid samples were stored under nitrogen in 1 mL chloroform-methanol (2:1, v/v) at -20°C until analyzed.

An aliquot of the erythrocyte membrane lipid sample from each animal was used for lipid and phospholipid class separation and determination using an Iatroscan TH-10 Mark II Analyser (T.M.A. Scientific Supply, Mississauga, Ont.), thin-layer chromatography (TLC)/flame ionization detection (FID) system (Newman 1978; Sipos and Ackman 1978; Tanaka *et al.* 1979; Van Tornout *et al.* 1979). Silica gel coated rods (Chromarods S-II, Technical Marketing Association Scientific Supply, Mississauga, Ont.) were used for the TLC separation of the lipid and phospholipid classes. Lipid samples (1-2 uL/rod) were spotted, in duplicate, on a set of 10 chromarods, developed for 1 h in chloroform-methanol-water (80:35:3.5) and scanned on the Iatroscan TH-10 Mark II Analyser for determination of lipid and phospholipid classes (Newman 1978; Appendix 6 and 7). Individual areas expressed as a percent of total peak area were measured with a Houston Model B5217 2-pen flat-bed chart recorder. Correction factors for chromarod and run variation and response of each lipid were determined and applied accordingly. An example of the determination and application of correction factors is given in Appendices 1, 2 and 3. Standard cholesterol and bovine phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, phosphatidylinositol and sphingomyelin

(Applied Science, Rexdale, Ont.) were used for lipid identification and determination of correction factors (Appendices 1, 2 and 3).

The remainder of the erythrocyte membrane lipid sample was used for TLC separation of cholesterol and the phospholipid classes. Total phospholipid, sphingomyelin and phosphatidylethanolamine were scraped from TLC plates, esterified and the fatty acid profiles of each determined by gas-liquid chromatography (GLC). Other erythrocyte membrane phospholipid classes were not analyzed for their fatty acid composition because of the impracticality of obtaining the large quantity of blood required for their accurate analyses. Further, sphingomyelin plus phosphatidylethanolamine account for approximately 90% of the phospholipids in cattle erythrocyte membranes. Both lipid and fatty acid percentages are calculated on a weight basis. The methods for TLC separation of the lipid classes, esterification of lipid classes and purification, separation (GLC) and identification of the fatty acids were as described by McClymont (1979) and Renner *et al.* (1979).

Throughout this chapter the fatty acids will be indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule.

Statistical analyses

The percentage of each lipid class and the percentage of individual fatty acids in each of the erythrocyte membrane total phospholipid, phosphatidylethanolamine and sphingomyelin fractions were statistically analyzed by least squares analyses of variance for unequal numbers (Harvey 1960). Sources of variation were phenotype (n=2), sex (n=2), age (n=2), their interactions and animals within phenotype x sex x age. Those sources of variation with significant F-values were subjected to the Student-Newman-Kuels multiple range test (Steel and Torrie 1980).

D. Results

Cholesterol was the only neutral lipid and phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine and sphingomyelin were the only phospholipids detected in the erythrocyte membranes from cattle of the DM breed group. Sphingomyelin was the predominant phospholipid found in the membranes accounting for 65 to 68% of the total phospholipid fraction. The mean concentrations of the lipid and phospholipid classes in erythrocyte membranes by sex, age and phenotype are presented in Table IV.1. Significant ($P<0.01$) differences were observed in the phospholipid class composition of erythrocyte membranes by sex and age. The percentage of sphingomyelin was significantly ($P<0.01$) lower in female as compared to male cattle while phosphatidylcholine was significantly (P<0.01) lower in the 9 to 22-mo age group as compared to the 33 to 46-mo age group. All other lipids expressed as a percentage of total lipid by sex and age for cattle of the DM breed group were not significantly (P>0.05) different. No significant (P>0.05) difference was observed in the erythrocyte membrane lipid class composition between phenotypically normal-muscled DM carriers and phenotypically extreme-muscled cattle.

The influence of sex on the total phospholipid, sphingomyelin and phosphatidylethanolamine fatty acid composition of erythrocyte membranes is reported in Table IV.2. Female animals had significantly (P<0.05) lower concentrations of 18:2 and 18:3 than male animals in all three phospholipid fractions. Erythrocyte membranes from the female animals also had significantly (P<0.01) increased concentrations of 14:1 and 16:0 in the total phospholipid fraction, significantly (P<0.05) decreased concentrations of 15:0, 15:1, 17:0 and 24:1 in the sphingomyelin fraction and significantly (P<0.05) increased concentrations of 18:0 and 24:0 and a significantly (P<0.05) decreased concentration of 24:1 in the phosphatidylethanolamine fraction. These differences are reflected in the significantly (P<0.01) increased concentration of saturated fatty acids and significantly (P<0.05) decreased concentration of polyunsaturated fatty acids of the phospholipid fractions in erythrocyte membranes from female as compared to male cattle.

Table IV.3 presents the influence of animal age on the fatty acid composition of the erythrocyte membrane total phospholipid, sphingomyelin and phosphatidylethanolamine fractions. The concentration of 18:3 and 22:5 were significantly ($P<0.05$) increased in the 33 to 46- as compared to the 9 to 22-mo age group for all three phospholipid fractions. The fatty acid pattern of total phospholipid also contained a significantly ($P<0.05$) lower concentration of 16:0, 17:0, 20:5, 24:0 and 24:1 and a significantly ($P<0.05$) higher concentration of 18:1 and 20:4 for the 33 to 46- as compared to the 9 to 22-mo age group. Erythrocyte membrane sphingomyelin from the older age group contained significantly ($P<0.05$) increased concentrations of 14:1, 15:0, 15:1, 20:5 and 24:0 and a significantly ($P<0.05$) decreased concentration of 18:1. Phosphatidylethanolamine from the 33 to 46-mo age group contained significantly ($P<0.05$) increased concentrations of 14:1 and 20:4 and significantly ($P<0.05$) decreased concentrations of 16:0, 16:1, 18:0 and 18:2 than from the 9 to 22-mo age group. A significantly ($P<0.05$) increased concentration of the polyunsaturated fatty acids was demonstrated for all three phospholipid fractions in erythrocyte membranes from the older age group. Only in the sphingomyelin fraction were the changes in the monounsaturated fatty acids consistent enough to result in a significantly ($P<0.05$) decreased proportion in the older as compared to the younger age group. The concentration of the total saturated fatty acids in the total phospholipid fraction for the older age group was significantly (P<0.01) decreased as compared to the younger age group.

The fatty acid composition of the erythrocyte membrane total phospholipid, sphingomyelin and phosphatidylethanolamine fractions by phenotype are reported in Table IV.4. The fatty acid composition of the total phospholipid fraction by phenotype did not differ significantly (P>0.05). However, significant (P<0.05) differences were observed in the fatty acid composition of the erythrocyte membrane major phospholipids by phenotype. Erythrocyte membrane sphingomyelin from phenotypically extreme-muscled DM cattle contained significantly (P<0.05) increased concentrations of 14:1, 15:0, 15:1, 16:0 and 18:3 and a significantly (P<0.01) decreased concentration of 18:1. These differences in fatty acid composition of sphingomyelin from extreme-muscled DM cattle are reflected in the significantly (P<0.05) increased concentrations of the saturated and polyunsaturated fatty acids and the significantly (P<0.01) decreased concentration of the monounsaturated fatty acids in these animals. The erythrocyte membrane phosphatidylethanolamine from extreme-muscled DM cattle had significantly (P<0.05) increased concentrations of 16:0, 18:0, 18:2 and 18:3 and a significantly (P<0.01) decreased concentration of 20:4 as compared to the phenotypically normal-muscled carriers. The total saturated and mono- and polyunsaturated fatty acid compositions in the phosphatidylethanolamine fraction by phenotype were not significantly (P>0.05) different. The total saturated and monounsaturated fatty acid composition in the erythrocyte membrane phosphatidylethanolamine by phenotype showed the same trend (P<0.1) as was observed in membrane sphingomyelin, although there were no differences in the relative amounts of polyunsaturated fatty acids.

E. Discussion

The lipid composition of erythrocyte membranes from cattle of the DM breed group observed in the present study are similar to those reported in the literature for cattle. Numerous workers (De Gier and Van Deenen 1961; Nelson 1967a,1967b,1972; Sweeley and Dawson 1969; O'Kelly and Mills 1979) have reported that the neutral lipid fraction of erythrocyte membranes from various species, including cattle, consists almost exclusively of cholesterol which accounts for 30 to 40% of the total lipid. Varying amounts of neutral lipids other than cholesterol had been reported in earlier studies but it is now generally agreed that these lipids arose from the failure to completely remove contaminants such as reticulocytes, leukocytes, platelets and plasma lipoproteins from the erythrocyte membrane preparation (Sweeley and Dawson 1969; Nelson 1972).

The erythrocyte membrane lipid of various species consists of approximately 60% phospholipid and in the case of cattle, sphingomyelin is the predominant phospholipid, with little or no phosphatidylcholine (De Gier and Van Deenen 1961; Nelson 1967b,1972; O'Kelly and Mills 1979). The phospholipid composition of erythrocyte membranes from all cattle in the present study were well within the range of those reported for cattle in the literature (De Gier and Van Deenen 1961; Nelson 1967b,1972; Van Deenen and De Gier 1974).

The fatty acid composition of the erythrocyte membrane total phospholipid and phosphatidylethanolamine from cattle in the present study are similar to the literature values for cattle (Nelson 1972; O'Kelly and Mills 1979) while that for sphingomyelin was somewhat different. Literature values for the fatty acid composition of erythrocyte membrane sphingomyelin from man as well as bovine are inconsistent, particularly for the relative concentrations of 18:0, 18:1, 18:2 and 24:0 (Rouser *et al.* 1968; Nelson 1972; O'Kelly and Mills 1979; Quinn and Chapman 1980). The reason for this discrepancy is unknown, though factors such as breed, age, sex, nutrition and animal type may be involved (Nelson 1972; O'Kelly and Mills 1979).

Theoretical values for the fatty acid composition of erythrocyte membrane total phospholipid fraction were determined by using the data from Table IV.1 and IV.4 and the fatty acid composition of cattle erythrocyte membrane phosphatidylserine and phosphatidylcholine reported by O'Kelly and Mills (1979). These theoretical values were compared to the observed fatty acid composition of erythrocyte membrane total phospholipid by phenotype (Table IV.4). The theoretical values are in relatively close agreement with the observed values for normal-muscled DM cattle. This was not so for the theoretical *versus* the observed fatty acid composition of erythrocyte membrane total phospholipid for extreme-muscled DM cattle, particularly in the case of 16:0 and 18:1. Two explanations may be proposed which may contribute to this discrepancy. The error involved in the calculation of these theoretical values is too great or the fatty acid composition of erythrocyte membrane phosphatidylserine and phosphatidylcholine in extreme-muscled DM cattle is greatly altered. The latter seems more plausible since the theoretical values are similar to the observed values for normal-muscled cattle.

Significant differences in the phospholipid class composition and the fatty acid composition of the phospholipids in erythrocyte membranes from female and male cattle of the DM breed group were observed in the present study. As far as the author is aware, no detailed studies have reported the influence of sex on erythrocyte membrane lipid class composition and phospholipid fatty acid composition from cattle. Sex related differences in the lipid composition of plasma lipoproteins (Skipski 1972) or hormone status (Grodsky 1977) may lead to differences in erythrocyte membrane lipid composition between sexes. Differences could arise during the *de novo* synthesis of erythrocytes (Nelson 1972) or later due to exchange of lipids between plasma lipoproteins and the erythrocyte membrane (Dise *et al*. 1980).

In the present study, many age-related changes were observed in the lipid composition of erythrocyte membranes from cattle of the DM breed group. In a previous study (Chapter II), the erythrocyte osmotic fragility was observed to decrease during aging in cattle of various breed groups. Changes in the lipid composition of the erythrocyte membrane are known to alter erythrocyte osmotic fragility (Kuiper *et al*. 1971; Sagawa and Shiraki 1978) as well as the fluidity and permeability of the erythrocyte membrane (Cooper 1969). Lower erythrocyte osmotic fragility has been associated with an increased erythrocyte membrane cholesterol/phospholipid ratio, with increased relative concentrations of cholesterol, phosphatidylcholine and sphingomyelin, and most profoundly, with an increased concentration of 18:3 accompanied by a decreased concentration of 16:0 (Kuiper *et al*. 1971; Sagawa and Shiraki 1978). The direction of change in the relative concentrations of erythrocyte membrane phosphatidylcholine, 18:3 and 16:0, in the present study, during aging are similar, suggesting that the lipid profiles observed in the present study may contribute to the decreased erythrocyte osmotic fragility associated with increasing animal age in cattle of the DM breed group.

Extreme-muscled DM cattle, in the present study, demonstrated significant differences in their fatty acid composition of the two major erythrocyte membrane phospholipid classes as compared to normal-muscled carriers of the "double-muscling" trait. In general terms, it is well documented that alterations in lipid and/or fatty acid composition of erythrocyte membranes influence membrane fluidity, permeability (Cooper 1969; Nelson 1972; Van Deenen and De Gier 1974; Grinna 1977), membrane-associated enzyme function, hormone receptor specificity (Masoro 1977) and osmotic fragility (Kuiper *et al.* 1971; Sagawa and Shiraki 1978). However, this does not attempt to explain the significance of the specific alterations in fatty acid composition of erythrocyte membranes observed for extreme-muscled DM cattle in the present study. Toward this end, it should be recalled that extreme-muscled DM cattle demonstrated an increased erythrocyte osmotic fragility as compared to carriers and to normal cattle (Chapter II). In the present study, extreme-muscled DM cattle demonstrated an 18% increase in 16:0 and a 20% decrease in 18:1 concentrations in their erythrocyte membrane sphingomyelin fraction and an 11% increase in 16:0 and a 5% decrease in 18:1 concentrations in their erythrocyte membrane phosphatidylethanolamine fraction as compared to normal-muscled carriers. In a study (Kuiper *et al.* 1971), in which the lipid composition of erythrocytes of a group of hamsters with increased erythrocyte fragility were compared to a group of hamsters with normal erythrocyte fragility, similar results were observed. For example, the erythrocyte membrane phospholipid classes from the group of hamsters with increased erythrocyte osmotic fragility demonstrated an average 25% increase in 16:0 and an average 27% decrease in 18:3 (Kuiper *et al.* 1971). In an interspecies comparison, Van Deenen and De Gier (1974) showed that an increased saturated and polyunsaturated and a decreased monounsaturated fatty acid composition in erythrocyte membranes is associated with increased permeability of glycerol and increased influx of phosphate. A similar pattern was observed in the fatty acid composition of erythrocyte membrane sphingomyelin and, to a lesser extent, phosphatidylethanolamine from extreme-muscled DM cattle. It may therefore be suggested that the increased susceptibility of erythrocytes to osmotic lysis in extreme-muscled DM cattle is due, in part, to the altered fatty acid composition of their erythrocyte membranes. It is also possible that these changes alter the fluidity and permeability and thus the functional state of their erythrocyte membranes. However, due to the great chemical heterogeneity of the membrane lipids (Van Deenen and De Gier 1974) and their complex interactions among themselves and with membrane proteins (Nelson 1972), it remains uncertain as to the overall influence the observed membrane structural alterations have on the functional integrity of erythrocyte membranes in double-muscled cattle.

The reason(s) for the changes in the fatty acid composition of erythrocyte membrane sphingomyelin and phosphatidylethanolamine observed in the present study for extreme-muscled DM cattle is (are) uncertain. Hulbert *et al.* (1976) have demonstrated that changes in thyroid status alter the lipid composition of membrane systems. The present author (Chapter III) and Strath *et al.* (1980) have demonstrated elevated serum triiodothyronine levels in extreme-muscled DM cattle as compared to carriers and normal cattle. The differences observed in the fatty acid composition of erythrocyte membranes by phenotype may also be due to differences in the overall animal metabolism of lipids. Erythrocytes do not contain the machinery for lipid synthesis and thus cannot alter fatty acid chain length or the degree of unsaturation or synthesize phospholipids *de novo* (Dise *et al.* 1980). Alterations in the fatty acid composition of erythrocyte membrane phospholipid may therefore be reflective of changes in lipoprotein lipid composition and possibly alterations in lipoprotein metabolism. However, it must be realized that specific enzymes are involved in exchange of lipids between plasma lipoproteins and erythrocyte membranes (Nelson 1972; Van Deenen and De Gier 1974; Dise *et al.* 1980). Differences in erythrocyte membrane lipid composition could therefore be due to the selectivity of enzymes incorporating specific types of phospholipids into the erythrocyte membrane and/or the incorporation of fatty acids into the membrane lipids. This point is made more obscure by the evidence which demonstrates that many membrane-associated enzymes are lipid-dependent (Masoro 1977) and changes in membrane lipid composition will ultimately influence enzyme function.

In conclusion, extreme-muscled DM cattle demonstrated alterations in the fatty acid composition of their erythrocyte membranes relative to normal-muscled carriers. These alterations may, in part, contribute to the increased erythrocyte osmotic fragility associated with this animal type. Further, the observed alterations may also infer changes in erythrocyte membrane fluidity and permeability and may be indicative of alterations in the lipid composition and functional state of other membrane systems in extreme-muscled DM cattle. The reason(s) for these differences is (are) unclear, though they may reflect changes in enzyme function or changes in cell metabolism possibly resulting from endocrine or neuroendocrine abnormalities in cattle exhibiting the Double Muscled Syndrome.

Table IV.1 Least squares means[1] of lipid composition of erythrocyte membranes by sex, age and phenotype in cattle of the DM breed group.

Lipids (% of total lipid[3])	Sex		Age (months)		Phenotype		SEM[2]
	Female	Male	9-22	33-46	Normal	Extreme	
Cholesterol	37.09	36.99	37.24	36.84	37.13	36.95	0.36
Phosphatidylethanolamine	14.86	14.08	14.48	14.47	14.37	14.57	0.40
Phosphatidylserine	5.53	5.06	5.51	5.08	5.49	5.09	0.19
Phosphatidylcholine	1.22	1.02	0.88	1.36**	1.06	1.18	0.10
Sphingomyelin	41.30	42.86**	41.90	42.26	41.94	42.21	0.36
Total Phospholipid	62.91	63.01	62.76	63.16	62.87	63.05	0.36

[1] Eleven animals per mean for the sex and phenotype effects. Twelve and ten animals per mean for the 9-22 and 33-46 month age groups, respectively.

[2] Standard error of the mean for sex and phenotype means. Average standard error of the mean for age means.

[3] Lipid percentages are calculated on a weight basis.

**Asterisks in each row within each major heading indicate a significant difference (P<0.01).

Table IV.2 Least squares means[1] of the percent fatty acid composition of the erythrocyte membrane total phospholipid, sphingomyelin and phosphatidylethanolamine by sex in cattle of the DM breed group.

Fatty acid (%)[2]	Total Phospholipid			Sphingomyelin			Phosphatidylethanolamine		
	Female	Male	SEM[3]	Female	Male	SEM[3]	Female	Male	SEM[3]
14:0	0.14	0.10	0.02	0.67	0.38	0.31	0.61	0.56	0.10
14:1	0.27	0.14	0.03*	0.19	0.26	0.06	0.99	0.51	0.17
15:0	0.22	0.18	0.02	0.25	0.46	0.05*	0.48	0.31	0.08
15:1	0.08	0.06	0.01	0.07	0.21	0.03**	0.21	0.17	0.04
16:0	10.08	8.31	0.40**	15.19	12.79	1.25	5.76	4.85	0.32
16:1	1.84	2.18	0.14	1.90	1.69	0.38	2.74	3.00	0.15
17:0	0.85	0.85	0.02	0.85	1.29	0.07**	0.36	0.30	0.04
18:0	15.83	16.31	0.35	24.01	21.87	1.65	5.48	4.87	0.15*
18:1	39.23	37.37	1.21	24.48	25.11	1.29	57.51	57.14	0.83
18:2	11.97	14.87	0.36**	8.29	9.98	0.38**	12.85	16.89	0.26**
18:3	0.81	1.65	0.13**	0.44	1.39	0.28*	0.99	1.77	0.14**
20:4	4.43	4.60	0.24	2.56	2.30	0.22	5.74	5.30	0.19
20:5	1.91	1.81	0.17	2.83	3.38	0.28	2.03	0.23	0.77
22:5	1.07	1.06	0.09	0.17	0.40	0.08	1.20	1.32	0.22
24:0	6.08	5.64	0.73	12.62	11.00	1.07	1.27	0.42	0.26*
24:1	1.98	2.00	0.32	2.24	3.50	0.20**	0.19	0.38	0.05*
Others[4]	3.21	2.88	0.28	3.24	3.99	0.41	1.58	1.99	0.27
Saturated	33.19	31.38	0.63	53.58	47.79	1.31**	13.96	11.31	0.59**
Monounsaturated	43.40	41.76	1.04	28.89	30.77	1.08	61.64	61.19	0.81
Polyunsaturated	20.19	23.98	0.62**	14.29	17.45	0.72**	22.82	25.51	0.68*

[1] Eleven animals per mean.

[2] Only predominant fatty acids, expressed as a percent of total fatty acids, are reported. Fatty acids are indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule. Fatty acid percentages are calculated on a weight basis.

[3] Standard error of least squares means. Asterisks in each row within each major heading indicate a significant sex difference: $*P<0.05$; $**P<0.01$

[4] The sum of ten fatty acids which were each less than one percent of total fatty acids.

Table IV.3 Least squares means[1] of the percent fatty acid composition of the erythrocyte membrane total phospholipid, sphingomyelin and phosphatidylethanolamine by animal age in cattle of the DM breed group.

Fatty acid (%)[2]	Total Phospholipid			Sphingomyelin			Phosphatidylethanolamine		
	9-22	33-46	SEM[3]	9-22	33-46	SEM[3]	9-22	33-46	SEM[3]
14:0	0.14	0.10	0.02	0.70	0.35	0.31	0.54	0.62	0.10
14:1	0.21	0.20	0.03	0.12	0.33	0.06*	0.48	1.02	0.17*
15:0	0.22	0.17	0.02	0.26	0.45	0.05*	0.48	0.32	0.08
15:1	0.08	0.07	0.01	0.08	0.21	0.03**	0.21	0.17	0.04
16:0	10.18	8.20	0.40**	14.17	13.81	1.25	6.06	4.54	0.32**
16:1	2.09	1.94	0.14	1.94	1.64	0.38	3.17	2.57	0.15*
17:0	0.91	0.79	0.02**	1.01	1.13	0.07	0.39	0.27	0.04
18:0	15.78	16.35	0.34	24.33	21.54	1.65	5.43	4.92	0.15*
18:1	36.25	40.34	1.21*	27.07	22.52	1.29*	58.09	56.55	0.84
18:2	13.15	13.69	0.36	9.12	9.15	0.38	15.52	14.23	0.26**
18:3	0.60	1.86	0.13**	0.37	1.46	0.28*	0.76	2.00	0.14**
20:4	3.95	5.08	0.24**	2.25	2.61	0.22	4.96	6.08	0.19**
20:5	2.21	1.51	0.17*	2.43	3.78	0.28**	0.18	2.08	0.76
22:5	0.88	1.25	0.09*	0.12	0.46	0.08*	0.83	1.69	0.22*
24:0	7.17	4.55	0.73*	10.00	13.62	1.06*	0.59	1.10	0.26
24:1	2.64	1.34	0.32*	2.99	2.74	0.20	0.22	0.34	0.04
Others[4]	3.54	2.55	0.28*	3.02	4.21	0.41	2.08	1.49	0.27
Saturated	34.40	30.17	0.63**	50.48	50.89	1.31	13.49	11.78	0.59
Monounsaturated	41.27	43.89	1.04	32.21	27.45	1.07**	62.18	60.66	0.81
Polyunsaturated	20.79	23.39	0.62*	14.29	17.45	0.72**	22.25	26.07	0.68**

[1] Twelve and ten animals per mean in the 9-22 and 33-46 month age groups, respectively.

[2] Only predominant fatty acids, expressed as a percent of total fatty acids, are reported. Fatty acids are indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule. Fatty acid percentages are calculated on a weight basis.

[3] Average standard error of least squares means. Asterisks in each row within each major heading indicate a significant age difference: $*P<0.05$; $**P<0.01$.

[4] The sum of ten fatty acids which were each less than one percent of total fatty acids.

Table IV.4 Least squares means[1] of the percent fatty acid composition of the erythrocyte membrane total phospholipid, sphingomyelin and phosphatidylethanolamine from phenotypically normal- and extreme-muscled DM cattle.

Fatty acid (%)[2]	Total Phospholipid			Sphingomyelin			Phosphatidylethanolamine		
	Normal	Extreme	SEM[3]	Normal	Extreme	SEM[3]	Normal	Extreme	SEM[3]
14:0	0.12	0.12	0.02	0.24	0.81	0.31	0.57	0.60	0.10
14:1	0.21	0.20	0.03	0.14	0.32	0.06*	0.70	0.80	0.17
15:0	0.20	0.19	0.02	0.14	0.57	0.05**	0.35	0.44	0.08
15:1	0.08	0.07	0.01	0.08	0.20	0.03**	0.18	0.21	0.04
16:0	9.71	8.68	0.40	11.50	16.47	1.25*	4.70	5.91	0.32*
16:1	2.10	1.93	0.14	1.56	2.03	0.38	2.74	2.99	0.15
17:0	0.85	0.84	0.02	0.97	1.17	0.07	0.33	0.33	0.04
18:0	15.93	16.20	0.35	24.30	21.58	1.65	4.89	5.46	0.15*
18:1	38.81	37.79	1.21	29.65	19.94	1.29**	58.56	56.09	0.83
18:2	13.26	13.58	0.36	8.63	9.64	0.38	14.05	15.69	0.26**
18:3	1.13	1.33	0.13	0.34	1.48	0.28*	0.98	1.78	0.14**
20:4	4.37	4.66	0.24	2.52	2.34	0.22	5.92	5.12	0.19**
20:5	1.84	1.88	0.17	2.72	3.49	0.28	1.91	0.35	0.77
22:5	1.00	1.13	0.09	0.26	0.32	0.08	1.18	1.34	0.22
24:0	5.70	6.03	0.73	10.88	12.74	1.07	0.92	0.77	0.26
24:1	1.81	2.17	0.32	2.67	3.06	0.20	0.27	0.29	0.05
Others[4]	2.90	3.20	0.28	3.39	3.84	0.41	1.75	1.82	0.27
Saturated	32.51	32.06	0.63	48.03	53.34	1.31*	11.76	13.51	0.59
Monounsaturated	43.00	42.16	1.04	34.10	25.56	1.08**	62.45	60.38	0.81
Polyunsaturated	21.60	22.58	0.62	14.48	17.26	0.72*	24.03	24.29	0.68

[1] Eleven animals per mean.

[2] Only predominant fatty acids, expressed as a percent of total fatty acids, are reported. Fatty acids are indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule. Fatty acid percentages are calculated on a weight basis.

[3] Standard error of least squares means. Asterisks in each row within each major heading indicate a significant phenotype difference: $*P<0.05$; $**P<0.01$.

[4] The sum of ten fatty acids which were each less than one percent of total fatty acids.

V. THE LIPID COMPOSITION OF PLASMA AND PLASMA LIPOPROTEINS FROM DOUBLE-MUSCLED CATTLE AND NORMAL-MUSCLED CARRIERS

A. Abstract

The fatty acid composition of the various lipid and phospholipid classes in the total plasma lipids, high density lipoproteins (HDL) and low plus very low density lipoproteins (LDL-VLDL) fractions were determined in six phenotypically extreme-muscled and six phenotypically normal-muscled cattle of a "Double-Muscled" (DM) breed group. There were two age groups and two sexes per experimental group. The phenotypically extreme-muscled DM cattle demonstrated a significantly decreased concentration of triacylglycerides in the plasma and HDL total lipid fractions and a significantly increased concentration of cholesterol in the plasma total lipid fraction as compared to phenotypically normal-muscled DM cattle. The concentration of triacylglyceride was decreased over two-fold, with a corresponding significant increase in the concentration of cholesterol esters in the LDL-VLDL total lipid fraction in extreme-muscled DM cattle as compared to phenotypically normal-muscled carriers. All other lipid classes in the total plasma, HDL and LDL-VLDL total lipid fractions were similar in composition for both phenotypes. All phospholipid classes, with the exception of lysophosphatidylcholine which was increased in the LDL-VLDL total lipid fraction from phenotypically extreme DM cattle

were similar in composition in the three total lipid fractions for both phenotypes. Extreme-muscled DM cattle demonstrated a significantly increased concentration of polyunsaturated fatty acids in the plasma and LDL-VLDL total lipid fractions, with a similar trend occurring in the HDL total lipid fraction. In each of these total lipid fractions, the enrichment in polyunsaturated fatty acids was due to an increased amount of linoleic acid (18:2) accompanied by a fall in the concentration of palmitic acid (16:0). The observed alterations in the lipid and fatty acid composition of plasma, HDL and LDL-VLDL total lipid fractions by DM phenotype are indicative of differences in the metabolism of serum lipoproteins between extreme-muscled DM cattle and normal-muscled carriers of the "double muscling" conformation.

B. Introduction

Serum lipoproteins facilitate the transport and exchange of various lipid constituents synthesized in the liver or intestine to peripheral tissues for storage and utilization (Eisenberg 1979; Steinberg 1979; Schaefer and Levy 1979). Very low-density lipoprotein (VLDL) particles are the major carriers of endogenously synthesized triacylglycerides (Eisenberg 1979). Through multi-step interactions of VLDL particles with endothelial bound lipoprotein lipases, the triacylglyceride moiety of these particles is removed, leaving a triacylglyceride-depleted, cholesterol ester-rich remnant or low-density lipoprotein (LDL) particle (Eisenberg 1979; Steinberg 1979). Animals with an increased energy demand increase their metabolism of VLDL particles (Raphael *et al*. 1973).

The metabolic rate of DM cattle has not been reported in the scientific literature. Kolataj *et al*. (1979a) measured the activity of certain plasma enzymes in extreme-muscled DM cattle and suggested that this animal type may have an increased metabolic rate. Pullar and Webster (1977) observed that lean rats produced more heat per unit body weight compared to fat Zucker rats. Hanset *et al*. (1979) speculated that, if the same were true in cattle, double muscled animals may have an increased maintenance requirement relative to normal animals of the same weight.

The following experimental evidence also supports the suggestion that cattle exhibiting the Double Muscled Syndrome have an increased energy demand and an altered lipoprotein metabolism. Double-muscled cattle are characterized by an increased potential for muscling and a decreased potential to deposit fat (Oliver and Cartwright 1968). The present author (Chapter III) and Strath *et al*. (1980) have shown that extreme-muscled DM cattle have an increased serum triiodothyronine concentration. King *et al*. (1976a) and Basarab *et al*. (1980; Chapter II) have demonstrated an increased erythrocyte osmotic fragility in extreme-muscled DM cattle as compared to carriers of the "double-muscling" trait and normal cattle. This result was suggested to be indicative of a generalized membrane defect (King *et al.* 1976a), and may possibly infer an increased energy demand for the maintenance of membrane integrity in double-muscled cattle. Further, a previous study (Chapter IV) had shown alterations in the fatty acid composition of erythrocyte membrane phospholipids from extreme-muscled DM cattle as compared to normal-muscled carriers of the "double-muscling" trait. One of the possible reasons suggested for this alteration was concerned with lipoprotein metabolism. Since erythrocytes do not contain the machinery for lipid synthesis and thus cannot alter fatty acid chain length or degree of unsaturation or synthesize phospholipids *de novo* (Dise *et al.* 1980), alterations in fatty acid composition of erythrocyte membrane phospholipids may be reflective of alterations in lipoprotein lipid composition and possibly alterations in lipoprotein metabolism.

The objective of the present study was therefore to examine the lipid metabolism in phenotypically extreme-muscled and normal-muscled cattle of a "Double-Muscled" (DM) breed group by determining the lipid composition of the plasma lipoproteins. These lipid profiles of plasma lipoproteins may also provide an insight into the reason for the structural lipid alterations in erythrocyte membrane in phenotypically extreme-muscled DM cattle.

C. Materials and Methods

Twelve cattle from a DM breed group, maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta, were used in this study. Six animals were subjectively categorized as phenotypically extreme- and six as phenotypically normal-muscled carriers, based on the degree of phenotypic expression of the "double-muscling" trait and their breeding histories (Chapter I). Three extreme-muscled and three normal-muscled male animals of 10 to 11-mo of age and three extreme-muscled and three normal-muscled female animals of 22 to 23-mo of age made up the two age-sex groups within each animal type. All animals were maintained under similar environmental conditions and were not fed 18 h prior to blood sample collection. Samples were obtained from the two age-sex groups at two different times, one week apart.

Blood sampling, lipoprotein separation and cholesterol determination

Each animal was restrained in a cattle squeeze and 60 mL of jugular blood were withdrawn, by venipuncture, into heparinized Vacutainers. The blood samples were immediately centrifuged at 1000 x g for 10 min at 20°C. The plasma was collected, quick frozen in liquid nitrogen and stored at -20°C until analyzed.

The precipitation of LDL plus VLDL particles from the plasma leaving the HDL particles in the supernatant was carried out according to a modified version of the sodium phosphotungstate-Mg^{2+} precipitation method described by Lopes-Virella *et al*. (1977). To 5 mL of plasma was added 500 uL of a solution of sodium phosphotungstate (5.0g phosphotungstic acid and 0.80g NaOH made to 100 mL with distilled water) and magnesium chloride (25.41g $MgCl_2.6H_2O$ made to 50 mL with distilled water). The combined solution was made immediately before use by mixing 4 mL of the sodium phosphotungstate solution and 1 mL of the magnesium chloride solution. All tubes containing plasma plus reagent were mixed using a vortex mixer and centrifuged at 1500 x g for 30 min at 2°C. Immediately after centrifugation, the clear supernatant containing the HDL fraction was transferred to another tube. Samples which did not yield a clear supernatant were diluted 1:1 with distilled water and re-precipitated. Aliquots of the whole plasma and the pooled HDL supernatant from each animal were quantitatively analyzed for total cholesterol using the enzymatic method adapted to the autoanalyzer (Technicon 1976). The calculation of HDL-cholesterol concentration (mg/dL) in plasma was performed using the formula: concentration of sample from standard curve x 1.1 = mg/dL HDL-cholesterol. In the case of a 1:1 dilution prior to precipitation, the calculation of HDL-cholesterol from this formula was multiplied by 2 to get final mg/dL of HDL-cholesterol. These cholesterol concentrations plus the percent lipid composition of plasma, HDL and LDL-VLDL were used for the calculation of total lipid concentrations in whole plasma, HDL and LDL-VLDL.

Lipid extraction and analyses

The lipid portion of the whole plasma, HDL and LDL-VLDL fractions per animal were twice extracted with chloroform-methanol (2:1, v/v) according to Folch *et al.* (1957). Lipid extractions on the LDL-VLDL fractions were carried out with homogenization (Polytron). The chloroform phases from each fraction were pooled, evaporated to dryness under nitrogen, and stored in 2 mL chloroform-methanol (2:1, v/v) under nitrogen at ⁻20°C. Aliquots of the whole plasma, HDL and LDL-VLDL lipid samples from each animal were used for lipid and phospholipid class separation and percent compositional determination using an Iatroscan TH-10 Mark II Analyser (T.M.A. Scientific Supply, Mississauga, Ont.), thin-layer chromatography (TLC)/flame ionization detection system (Newman 1978; Sipos and Ackman 1978; Tanaka *et al.* 1979; Van Tornout *et al.* 1979). Silica gel coated rods (Chromarods S-II, Technical Marketing Association, Scientific Supply,Mississauga. Ont.) were used for the TLC separation of the lipid and phospholipid classes. Each lipid sample (1-2 uL/rod) was spotted on a chromarod, developed for 45 min in methylene chloride-chloroform-acetic acid-methanol (98:8:0.4:0.15) for lipid classes separation (Appendix 7) and scanned on the Iatroscan TH-10 Mark II Analyser to determine the relative amount of each lipid class (Newman 1978). The rods were re-spotted, developed in the above described solvent system, scanned to the phospholipid band, re-developed for 1 h in chloroform-methanol-water (80:30:3.5) and re-scanned completely for the determination of the relative amount of each phospholipid class (Appendix 8; Newman 1978). All determinations were carried out in duplicate. Peak percentages as a percent of total peak area were measured with a Houston Model B5217 2-pen flat-bed chart recorder. Correction factors (Appendices 1, 2 and 3) for chromarod and run variation and response of each lipid were determined and applied accordingly. Lipid standards (Sigma) of cholesterol esters, triacylglycerides, free fatty acids, unesterified cholesterol and phospholipids (phosphatidylcholine and sphingomyelin, 4:1, w/w) and phospholipid standards (Applied Science, Rexdale, Ont.) of bovine phosphatidylethanolamine, phosphatidylserine, phosphatidylcholine, phosphatidylinositol, sphingomyelin and lysophosphatidylcholine were used for lipid identification and determination of correction factors (Appendices 1, 2 and 3).

The remainder of each total lipid sample from each of the whole plasma, HDL and LDL-VLDL fractions were used for the determination of total lipid fatty acid profiles by gas-liquid chromatography (GLC). Both lipid and fatty acid percentages were calculated on a weight basis. The methods for the esterification of total lipid, purification of methyl esters, separation (GLC) and identification of the fatty acids are as described by McClymont (1979).

Throughout this chapter the fatty acids will be indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule.

Statistical analyses

The lipid and phospholipid relative concentrations, the total lipid concentration and the fatty acid percentage compositions of total lipid in each of the plasma, HDL and LDL-VLDL fractions were statistically analyzed using analysis of variance (Steel and Torrie 1980). Sources of variation were phenotype (n=2), age-sex (n=2), phenotype x age-sex and animals (n=3) within phenotype x age-sex. The error term for all sources of variance was animals within phenotype x age-sex. The age-sex combination was intentionally confounded so as to allow removal of variation due to this combination in the analyses of variance. The age-sex combinations resulted because of the kinds of animals available and were included in order to increase the number of animals available for testing the effect of phenotype.

D. Results

Table V.1 presents the total lipid concentration and the lipid and phospholipid class composition of the plasma and the HDL and LDL-VLDL total lipid fractions for cattle of the DM breed group. The major lipids in plasma and in the

HDL fraction were cholesterol esters and phospholipids, with the same two lipids plus triacylglycerides being the major lipids in the LDL-VLDL fraction. The HDL fraction accounted for 62-67% of the total plasma lipid with LDL-VLDL accounting for the remainder. Phosphatidylcholine accounted for 70-80% of the total phospholipids, with sphingomyelin being secondary, in the plasma, HDL and LDL-VLDL total lipid fractions.

The effect of phenotype was demonstrated to significantly ($P<0.05$) influence the lipid class composition of plasma, HDL and LDL-VLDL total lipid fractions (Table V.1). The relative concentration of triacylglycerides in plasma, HDL and LDL-VLDL total lipid fractions was significantly ($P<0.05$) decreased in phenotypically extreme-muscled DM cattle as compared to phenotypically normal-muscled carriers. By far the greatest ($P<0.01$) difference in the relative amount of triacylglycerides was observed in the LDL-VLDL total lipid fraction, where phenotypically extreme-muscled DM cattle demonstrated a two-fold decrease in the relative concentration of triacylglyceride as compared to phenotypically normal-muscled carriers. The relative concentration of cholesterol in plasma total lipid and that of cholesterol esters in LDL-VLDL total lipid were significantly ($P<0.05$) increased in phenotypically extreme-muscled DM cattle. The relative concentration of all other lipid classes in the plasma, HDL and LDL-VLDL total lipid fractions and the total lipid concentration of these fractions by phenotype were not observed to differ significantly ($P>0.05$).

The relative amounts of the major phospholipid classes in plasma, HDL and LDL-VLDL by phenotype were not found to be significantly ($P>0.05$) different (Table V.1). The relative concentration of lysophosphatidylcholine in the LDL-VLDL fraction was significantly ($P<0.05$) increased in phenotypically extreme-muscled DM cattle as compared to normal-muscled carriers of the "double-muscling" conformation.

The mean percent fatty acid composition of plasma, HDL and LDL-VLDL total lipid fractions by phenotype are presented in Table V.2. In all fractions, linoleic acid (18:2) was the predominant fatty acid, with oleic acid (18:1), stearic acid (18:0) and palmitic acid (16:0) being secondary. Significant ($P<0.05$) alterations in the percent fatty acid composition of the plasma and LDL-VLDL fractions were demonstrated between phenotypes. For example, the concentration of 16:0 in the plasma was significantly ($P<0.05$) decreased in phenotypically extreme-muscled DM cattle as compared to normal-muscled carriers. The same trend ($P<0.1$) was observed for the concentration of 16:0 in the HDL and the LDL-VLDL fractions. The relative amount of 18:2 in plasma and LDL-VLDL fractions was significantly ($P<0.05$) increased in phenotypically extreme-muscled DM cattle as compared to normal-muscled carriers, with the same trend ($P<0.1$) occurring in the HDL total lipid. These differences in fatty acid composition are reflected in the trend ($P<0.1$) toward decreased relative concentrations of saturated fatty acids in the HDL and LDL-VLDL total lipid fractions, in the significantly ($P<0.05$) increased relative concentrations of polyunsaturated fatty acids in the plasma and LDL-VLDL fractions and in the trend ($P<0.1$) toward an increased relative concentration of polyunsaturated fatty acids in the HDL fraction in phenotypically extreme-muscled DM cattle as compared to normal-muscled carriers. The relative amounts of all other fatty acids were not observed to differ significantly ($P>0.05$) in the plasma, HDL and LDL-VLDL fractions by phenotype.

E. Discussion

The quantity of lipid associated with the lipoprotein fractions and the lipid composition of plasma and of the HDL fraction for cattle in the present study were similar to those reported by others for ruminants (Evans *et al*. 1961; Duncan and Garton 1962; Garton and Duncan 1964; Mills and Taylaur 1971; Nelson 1973; Raphael *et al*. 1973). Cholesterol esters are the primary neutral lipid accounting for 55-65%, while the phospholipids account for 25-30% of the total lipid in plasma and in the HDL fraction. Triacylglycerides are a minor component of both plasma and the HDL fraction accounting for 2-7% of their total lipid. In cattle and sheep, the total lipid of the LDL fraction represents 20-40% of the total plasma lipid (Puppione *et al*. 1970; Dryden *et* *al.* 1971; Raphael *et al.* 1973; Wendlandt and Davis 1973; Calvert 1976) and is composed of 45-55% cholesterol esters, 25-35% phospholipids, 10-12% cholesterol, 5-10% triacylglycerides and less than 1% free fatty acids (Mills and Taylaur 1971; Nelson 1973). The total lipid of the VLDL fraction, on the other hand, represents less than 5% of the total plasma lipid (Puppione *et al.* 1970; Dryden *et al.* 1971; Raphael *et al.* 1973; Wendlandt and Davis 1973; Calvert 1976) and is composed of primarily triacylglycerides (55-65%), with lesser amounts (20%) of each of cholesterol and cholesterol esters (Nelson 1973; Raphael *et al.* 1973). On the basis of these values for the lipid composition of the LDL and VLDL fractions for cattle and sheep, a theoretical lipid composition of a combined LDL and VLDL fraction for cattle was calculated . The theoretical values are in agreement with the observed lipid composition of the LDL-VLDL fraction for cattle in the present study. Variations in the literature values reported for the lipid composition of plasma and plasma lipoproteins are primarily due to variation in the physiological and nutritional state of the animal (Duncan and Garton 1963; Dryden *et al.* 1971; Marchello *et al.* 1971; Raphael *et al.* 1973).

The phospholipid distribution in total plasma and in the lipoprotein fractions from cattle of the DM breed group were similar to those reported for other ruminants (Nelson 1967c,1969,1973), as well as man (Skipski 1972), in which phosphatidylcholine was the predominant phospholipid

(65-75%) and choline-containing phospholipids accounted for 90-95% of total phospholipids. Phosphatidylserine in this study as well as in others (Nelson 1967c, 1969, 1973; Skipski 1972) was a minor component of plasma lipoproteins accounting for less than 1%. Nelson (1969) suggests that because of the net negative charge on phosphatidylserine at physiological pH, it may not be useful in forming soluble lipoproteins.

The fatty acid profiles of plasma total lipid for cattle in the present study were generally similar to those reported by Evans *et al.* (1961) and Nelson (1969) for cattle and sheep, in which 18:2 was the major fatty acid, and 18:1, 18:0 and 16:0 each accounted for 10-20% of total fatty acid. Garton and Duncan (1964) and Leat (1966) reported a high concentration of linolenic acid (18:3) in bovine plasma total lipid. However, Dryden *et al.* (1971) and Marchello *et al.* (1971) subsequently demonstrated that 18:3 is a minor fatty acid in bovine plasma and plasma lipoprotein total lipid. Further, Dryden *et al.* (1971) demonstrated that the fatty acid profiles of the lipid classes of HDL and LDL were generally not greatly different from serum or each other. The results presented by Dryden *et al.* (1971) and Marchello *et al.* (1971), when calculated to the fatty acid percent composition of plasma, HDL and LDL total lipid, were generally similar to the fatty acid profiles of plasma, HDL and LDL-VLDL total lipid in the present study.

Extreme-muscled DM cattle demonstrated a two-fold decrease in the relative concentration of triacylglycerides in the LDL-VLDL total lipid fraction as compared to normal-muscled carriers of "double-muscling". This difference may be largely attributed to the VLDL particles since, in the bovine as well as in man and various other mammalian species (Mills and Taylaur 1971; Nelson 1973; Raphael *et al.* 1973; Jackson *et al.* 1976; Eisenberg 1979), they are the major transport vehicles for endogenously synthesized triacylglycerides from the liver. Further, through multi-step interactions of VLDL particles with endothelial bound lipoprotein lipases, the triacylglyceride moiety of these particles are removed leaving a triacylglyceride-depleted, cholesterol-rich remnant or LDL (Stead *et al.* 1978; Eisenberg 1979; Steinberg 1979). Thus, the difference observed in the present study in the relative concentration of cholesterol esters in LDL-VLDL total lipid by phenotype may be largely attributed to the LDL particles. These results suggest that extreme-muscled DM cattle have an increased rate of withdrawal of triacylglycerides from the VLDL particles and thus an increased conversion of VLDL particles to LDL particles. If the concentration of LDL to VLDL particles was increased in extreme-muscled DM cattle, this could account for the decreased relative concentration of triacylglycerides and increased relative concentration of cholesterol esters in the LDL-VLDL fraction of this animal type. Further, an increased conversion of VLDL to LDL particles and/or a preferential withdrawal of VLDL- and/or LDL-triacylglycerides could also account for the decreased relative concentration of triacylglycerides observed in the HDL total lipid fraction from extreme-muscled DM cattle, since transfer of lipids and proteins between VLDL and HDL particles and LDL and HDL particles has been demonstrated (Glomset 1979; Schaefer and Levy 1979). The decreased relative concentration of triacylglycerides in plasma total lipid from extreme-muscled DM cattle reflects those differences observed in their lipoproteins.

Raphael *et al.* (1973) found decreased levels of VLDL lipids and increased levels of LDL and HDL lipids during peak-lactation and lactating-pregnancy in cows as compared to the same animals during non-lactating, non-pregnancy periods. These authors suggested that this result could be reflective of an increased metabolism or turnover of VLDL particles in lactating-pregnant cows. Decreased relative concentrations of LDL and HDL triacylglycerides for cows during lactation and pregnancy as compared to the same cows during the dry period were also demonstrated by Raphael *et al.* (1973). This result is suggestive of a preferential use of triacylglycerides, particularily of VLDL origin, during periods of increased energy requirement. The finding that adiposity and relative body weight correlates directly with plasma triacylglycerides (Witztum and Schonfeld 1979) tends to support this suggestion. Extreme-muscled DM cattle apparently have an increased energy demand, as indicated by their increased muscularity and decreased fatness (Oliver and Cartwright 1968), increased serum triiodothyronine concentration (Chapter III; Strath *et al.* 1980) and altered functional state of their erythrocyte membranes (Chapter II and IV). Recently, Strath *et al.* (1981) have demonstrated an increased metabolic rate in extreme-muscled DM cattle relative to carriers. Thus, it may be suggested that triacylglycerides, particularily of VLDL origin, are withdrawn and utilized at a greater rate in extreme-muscled DM cattle as compared to normal-muscled carriers of "double-muscling". Further, an increased withdrawal of VLDL-triacylglycerides would result in an increased conversion of VLDL to LDL particles or an increased turnover of VLDL particles in extreme-muscled DM cattle.

The fatty acid profiles of the plasma and the lipoprotein total lipid fractions observed in the present study also support the suggestion of an increased turnover of lipoproteins in extreme-muscled DM cattle. Extreme-muscled DM cattle demonstrated a 51% ($P<0.01$), 20% ($P<0.1$) and 23% ($P<0.05$) increase in the relative concentration of the polyunsaturated fatty acids in LDL-VLDL, HDL and plasma total lipid fractions, respectively, as compared to phenotypically normal-muscled carriers. In each of these total lipid fractions, the enrichment in polyunsaturation was due to an increased 18:2 concentration accompanied by a fall in 16:0 concentration. Shepherd *et al.* (1978) have shown that these fatty acid compositional changes in man are associated with an increased HDL fluidity and suggested that alterations in fluidity influence lipoprotein catabolism. Results of previous studies also support the suggestion that changes in the fatty acid composition of lipoproteins alters their rate of catabolism. For example, Thompson *et al.* (1976) demonstrated that increased saturation of LDL cholesterol esters decreased LDL apolipoprotein catabolism. Soutar *et al.* (1974) showed that lecithin:cholesterol acyl transferase, an enzyme involved in lipoprotein metabolism, was dependent on the fatty acid composition of the substrate. Furthermore, triacylglyceride lipase, another enzyme involved in lipoprotein metabolism, hydrolyzed unsaturated triacylglycerides faster than saturated ones (La Rosa *et al.* 1972). This evidence demonstrates that enzymatic reactions with lipid substrates are influenced by the fluidity of the lipids involved and that lipoproteins which exhibit alterations in fatty acid composition which change their fluidity will influence the rate at which these particles are catabolized. However, if both phenotypic groups in the present study ate the same ration, why would extreme-muscled DM cattle have an increased proportion of polyunsaturated fatty acids? The most plausible reason for this increased amount of polyunsaturated fatty acids probably and simply reflects the increased proportion of cholesterol esters and phospholipids in plasma and plasma lipoproteins from extreme-muscled DM cattle as compared to normal-muscled carriers. Cholesterol esters, in particular, and phospholipids in plasma and plasma lipoproteins in cattle and sheep are much more polyunsaturated than triacylglycerides (Evans *et al.* 1961; Duncan and Garton 1962, 1963; Garton and Duncan 1964; Leat 1966; Moore *et al.* 1968; Nelson 1969; Dryden *et al.* 1971; Marchello *et al.* 1971). Thus, the increased polyunsaturation in plasma lipoproteins from extreme-muscled DM cattle merely reflects their increased utilization of triacylglycerides rather than mediating an increased metabolism of their lipoproteins.

One of the reasons for conducting this study was that it may provide insight into the structural lipid alterations in erythrocyte membranes in extreme-muscled DM cattle (Chapter IV). Though an increased polyunsaturation was observed in erythrocyte membrane phospholipids and in lipoprotein lipids from extreme-muscled DM cattle, other alterations in the fatty acid composition of lipoprotein lipids were observed which were not similar. This suggests that the changes observed in the fatty acid composition of erythrocyte membrane phospholipids in extreme-muscled DM cattle is due to some other mechanism. However, it should be realized that the manner in which phospholipids and phospholipid fatty acids are exchanged between lipoproteins and the erythrocyte membranes is not completely known (Dise *et al.* 1980).

In conclusion, the results of the present study suggest that extreme-muscled DM cattle utilize lipoprotein triacylglycerides at an increased rate because of their increased demand for energy. This proposed increased triacylglyceride utilization may be mediated through increased metabolic activities of lipoprotein lipases possibly resulting from changes in endocrine or neuroendocrine function.

Table V.1 Mean[1] lipid and phospholipid class composition of plasma, high density lipoprotein (HDL) and low-very low density lipoprotein (LDL-VLDL) lipid from phenotypically normal- and extreme-muscled DM cattle.

	Plasma			HDL			LDL-VLDL		
Lipid (% of total lipid[3])	Normal	Extreme	SEM[2]	Normal	Extreme	SEM[2]	Normal	Extreme	SEM[2]
Cholesterol esters	58.03	60.00	1.97	60.39	63.72	2.62	43.99	57.31	2.87*
Triacylglycerides	6.96	3.91	0.83*	1.29	0.22	0.22*	26.36	12.98	2.57**
Free Fatty Acids	2.75	1.46	0.83	2.84	1.44	0.48	0.94	0.75	0.22
Cholesterol	4.84	5.38	0.12*	4.82	4.75	0.15	5.93	5.64	0.43
Phospholipid	27.42	29.25	1.33	30.66	29.87	2.16	22.75	23.32	1.35
Total Lipid (mg/dL)	145.45	162.92	14.25	96.88	100.82	7.22	59.55	58.72	13.60
Phospholipid (% of total phospholipid[3])									
Phosphatidylethanolamine	2.21	0.83	0.51	3.17	1.72	0.62	2.12	4.33	2.04
Phosphatidylserine	0.94	0.81	0.23	0.56	0.46	0.14	1.90	1.56	0.44
Phosphatidylcholine	76.40	77.90	3.00	76.71	80.83	1.88	75.57	71.87	4.01
Sphingomyelin	16.64	15.48	1.78	14.67	13.12	1.68	18.02	18.03	2.11
Lysophosphatidylcholine	3.80	4.98	1.19	4.89	3.87	0.52	2.38	4.21	0.47*

[1] Six animals per mean.

[2] Standard error of the mean. Asterisks in each row within each major heading indicate a significant phenotype difference: $*P<0.05$; $**P<0.01$

[3] Lipid percentages are calculated on a weight basis.

Table V.2 Mean[1] fatty acid composition of plasma, high density lipoprotein (HDL) and low-very low density lipoprotein (LDL-VLDL) lipid from phenotypically normal- and extreme-muscled DM cattle.

Fatty acid (%)[2]	Plasma			HDL			LDL-VLDL		
	Normal	Extreme	SEM[3]	Normal	Extreme	SEM[3]	Normal	Extreme	SEM[3]
14:0	0.91	0.53	0.20	0.57	0.60	0.08	1.30	0.67	0.21
14:1	1.23	1.09	0.12	0.89	1.24	0.14	1.43	1.14	0.13
15:0	1.00	0.84	0.14	0.76	0.99	0.08	1.85	0.98	0.47
15:1	0.43	0.50	0.12	0.43	0.46	0.06	0.81	0.46	0.26
16:0	14.77	12.74	0.54*	13.26	11.30	0.72	17.77	14.57	1.00
16:1	3.44	2.54	0.38	2.98	3.63	0.35	3.76	2.43	0.55
17:0	1.48	1.79	0.10	1.42	1.85	0.16	1.76	1.98	0.12
18:0	18.71	17.26	1.45	16.08	14.00	0.95	23.93	20.00	2.02
18:1	16.49	13.52	1.31	16.74	13.51	1.46	16.53	13.88	1.50
18:2	30.83	36.28	1.58*	31.52	37.98	2.02	21.76	31.14	1.47**
18:3	4.10	6.70	1.13	5.13	6.08	0.68	2.42	5.34	1.06
20:4	1.99	2.27	0.32	2.84	3.28	0.35	1.74	2.54	0.41
Others[4]	4.63	3.95	0.85	7.38	5.08	1.30	4.94	4.85	0.88
Saturated	36.86	33.16	1.86	32.09	28.75	1.11	46.60	38.21	2.71
Monounsaturated	21.59	17.64	1.57	21.04	18.83	1.69	22.54	17.91	1.95
Polyunsaturated	36.92	45.24	2.44*	39.49	47.33	2.64	25.92	39.03	2.29**

[1] Six animals per mean.

[2] Only predominant fatty acids, expressed as a percent of total fatty acid, are reported. Fatty acids are indicated by the general shorthand form of XX:X, where XX indicates the number of carbon atoms in the molecule and X after the colon indicates the number of double bonds in the molecule. Fatty acid percentages are calculated on a weight basis.

[3] Standard error of least squares mean. Asterisks in each row within each major heading indicate a significant phenotype difference: *P<0.05; **P<0.01

[4] The sum of eleven fatty acids which were each less than one percent of total fatty acid.

VI. GLUCOSE CONSUMPTION AND LACTIC ACID PRODUCTION BY ERYTHROCYTES FROM DOUBLE-MUSCLED CATTLE, CARRIERS AND NORMAL CATTLE

A. Abstract

The *in vitro* glucose consumption and lactic acid production by erythrocytes from 20 cattle of a Beef Synthetic (SY) and 25 cattle of a "Double-Muscled" (DM) breed groups were determined. There were three age groups and two sexes within each breed group. Animals within the DM breed group were categorized as phenotypically normal- to moderate-muscled and extreme-muscled based on the phenotypic expression of the "double-muscling" trait and on their breeding history. Animal age was not found to significantly influence glucose consumption or lactic acid production by erythrocytes. Both glucose consumption and lactic acid production by erythrocytes from cattle of the DM breed group were significantly increased as compared to that by erythrocytes from cattle of the SY breed group. Extreme-muscled DM cattle were not significantly different in either their erythrocyte glucose consumption or lactic acid production compared to carriers of the "double-muscling" trait. Both extreme-muscled DM cattle and carriers demonstrated significantly increased erythrocyte glucose consumption and lactic acid production relative to normal-muscled non-carrier cattle of the SY breed group. No significant difference was observed in the erythrocyte

lactic acid production to glucose consumption molar ratios by breed group or by phenotype. This increased rate of erythrocyte glycolysis observed for carriers of the Double Muscled Syndrome, regardless of phenotypic expression of the "double-muscling" trait, may be in response to the maintenance of the integrity of an abnormal, possibly more fragile or "leaky" erythrocyte membrane.

B. Introduction

King and associates (King 1975; King *et al*. 1976a) were the first to demonstrate an increased erythrocyte osmotic fragility in cattle exhibiting the Double Muscled Syndrome (DMS). More recently, Basarab *et al*. (1980) observed an increased susceptibility of erythrocytes to osmotic lysis from phenotypically extreme double-muscled (DM) cattle as compared to normal-muscled non-carrier cattle (Chapter II). These workers (King *et al*. 1976a; Basarab *et al*. 1980) suggested that cattle exhibiting DMS may have an erythrocyte membrane alteration which may be indicative of a more generalized membrane defect in these animal types. The nature of the abnormality resulting in an increased erythrocyte osmotic fragility in DMS cattle is unknown. However, the present author had previously observed significant differences in the fatty acid composition of erythrocyte membrane phospholipids between extreme-muscled DM cattle and normal-muscled carriers of the "double-muscling" trait (Chapter IV). Cooper (1969) and

Grinna (1977) have reported that alterations in the fatty acid composition of erythrocyte membranes can alter erythrocyte osmotic fragility and erythrocyte membrane fluidity and permeability. The present author had also observed significantly increased serum triiodothyronine levels and a trend toward increased serum thyroxine levels in phenotypically extreme-muscled DM cattle as compared to normal-muscled, non-carrier cattle (Chapter III). Hulbert *et al.* (1976) have shown that thyroid hormones alter membrane lipid composition and hence their fluidity. Other workers (Necheles and Beutler 1959; Snyder *et al.* 1971; Monti and Wadso 1976a) have demonstrated that thyroid hormones significantly increased erythrocyte glucose metabolism. Thus, it is unclear whether the increased erythrocyte osmotic fragility observed in extreme-muscled DM cattle was due to an alteration in some structural component of their erythrocyte membrane or may reflect an alteration in erythrocyte metabolism possibly resulting from an endocrine alteration.

Glucose metabolism in mature erythrocytes occurs primarily by way of the Embden-Meyerhof pathway with 5-10% metabolized by the pentose phosphate pathway (Rapoport 1968; Kaneko 1974). The metabolism of glucose by these pathways results in the formation of products (ATP, NADH, NADPH, etc.) which are intimately involved in maintaining the integrity of the erythrocyte membrane (Kaneko 1974). Thus changes in glucose metabolism may either result in or reflect alterations in the functional state of the erythrocyte membrane. Therefore, the objective of the present study was to compare the rates of erythrocyte metabolism in extreme-muscled DM cattle, carriers of the "double-muscling" trait and normal-muscled non-carrier cattle by determining the rate of glucose consumption and lactic acid production in their erythrocytes.

C. Materials and Methods

Twenty cattle of a Beef Synthetic (SY) breed group and 25 cattle of a DM breed group maintained at The University of Alberta Beef Research Ranch, Kinsella, Alberta, were used in the present study. There were two sexes and three age groups (17 to 18-, 29 to 30- and 41 to 42-mo of age) within each breed group. Animals within the DM breed group were scored on a subjective scale of 1 (normal-muscling) to 5 (extreme-muscling) based on the degree of phenotypic expression of the "double-muscling" trait. Animals within the DM breed group receiving a score of 1 to 4 and 5 were categorized as phenotypically normal-to moderate- and extreme-muscled, respectively. All animals in the present study categorized as normal- to moderate-muscled were assumed to be carriers of the "double-muscling" trait since they had either produced double-muscled calves or were sired by an extreme-muscled bull (Chapter I). The SY breed group was included as a normal-muscled, non-carrier group, which showed greatest similarity in breed composition and muscling- and fattening-type to the DM breed group (Chapter I; Basarab *et al*. 1980).

Preparation and incubation of whole blood.

Each animal was restrained in a cattle squeeze and 60 mL of jugular blood were withdrawn by venipuncture into heparinized Vacutainers. To a 50 mL aliquot of the whole blood was added 450 uL of an isotonic Tris buffer (0.172, pH 7.4), isotonic glucose solution (5.51%). A preliminary study had shown that during a 4 h incubation of erythrocytes, glucose may become limiting. The whole blood solution, containing 2.73 mM additional glucose was mixed thoroughly. Five mL aliquots were transferred to nine sterile 10 mL, screw-capped, glass tubes and incubated at 37°C in a shaking water bath for 4 h. All samples were prepared for incubation within 10 to 15 min of blood collection. Aseptic technique was observed throughout the preparation of whole blood for the *in vitro* incubation. At zero time and at subsequent 30 min intervals single tubes were removed from the incubation bath and inverted several times prior to duplicate hematocrit determinations. A 2 mL aliquot of whole blood was immediately withdrawn and slowly run into 4 mL cold 8% perchloric acid to stop glycolysis. This mixture was vortexed for 30 sec and left in an ice water bath for 5 min prior to centrifugation at 3000 x g for 10 min at 20°C. The protein-free supernatant was refrigerated at 4°C until used for lactic acid analysis. The remaining blood was centrifuged for 10 min at 3000 x g, the plasma collected, quick frozen in dry ice and stored at $^{-}20°C$ until analyzed for glucose.

Hematocrits were determined by a standard microhematocrit method. Lactic acid was determined by a commercial lactic acid kit (Sigma Chemical Co., No.826-UV) using the principle of enzymatic conversion of lactate to pyruvate and the reduction of NAD to NADH which was measured on a Gilford 240 spectrophotometer at 340nm. Plasma glucose was analyzed by the neocuproine glucose method (Bittner and Manning 1967) adapted for the autoanalyzer (Technicon method No.SE4-0002FF4).

Calculations and statistical analyses.

Glucose concentrations (mmoles/L plasma) at each 30 min interval per animal throughout the 4 h incubation were converted to mmoles/L packed cells as follows (Love *et al.* 1977):

Glucose (mmoles/L packed cells) = Glucose (mmoles/L plasma) x total plasma volume(100-PCV)/total cell volume (PCV).

Lactic acid concentrations (mmoles/L blood) for each time interval per animal over the 4 h incubation divided by PCV/100 gives mmoles lactic acid/L packed cells. Each glucose and lactic acid calculation was done using the individual PCV value from the appropriate incubation time interval. The difference between the glucose or lactic acid concentrations (mmoles/L packed cells) per animal at the beginning and end of each incubation time interval represents the amount of glucose consumed or lactic acid produced per incubation time interval. Glucose consumption and lactic acid production rates (mmoles/L packed cells/h) for each animal were then determined by the linear regression of mmoles lactic acid or glucose/L packed cells against incubation time intervals.

The glucose consumption and lactic acid production rates, the initial plasma glucose and blood lactic acid concentrations, the initial PCV value and the lactic acid production to glucose consumption (mmoles/L packed cells/h) molar ratios per animal were statistically analyzed by least squares analysis of variance (Harvey 1960). Sources of variance for the testing of breed groups were sex (n=2), age (n=3), sex x age, breed group (n=2), sex x breed groups and age x breed groups. Sources of variance for the testing of phenotypes were sex, phenotype (n=3), sex x phenotype, age, sex x age and phenotype x age. Those sources of variance with significant F-values were tested for significance by the Student-Newman-Keuls multiple comparison of means (Steel and Torrie 1980).

D. Results and Discussion

The least squares mean initial plasma glucose concentration of 9.25±0.17mM observed for cattle of the SY breed group was significantly (P<0.01) higher than the mean of 7.68±0.14mM observed for cattle of the DM breed group.

Analysis of the data by phenotype showed that initial glucose concentrations between extreme-muscled DM cattle and phenotypically normal-to moderate-muscled cattle of the DM breed group were not significantly (P>0.05) different (Table VI.1). Both DM phenotypes gave mean initial glucose concentrations significantly (P<0.01) lower than that observed in normal-muscled non-carrier SY cattle (Table VI.1). Taking into consideration that an average of 4.38 mmole glucose/L plasma was added to each sample prior to incubation, the actual plasma glucose concentration observed for cattle in the present study are within the range of those reported by others (Holmes and Robinson 1970; Holmes *et al.* 1973; Strath *et al.* 1980).

The least squares mean initial blood lactic acid concentration of 2.66±0.49 mM observed for SY cattle was not significantly (P>0.05) different from the mean of 1.94±0.42 mM observed for cattle of the DM breed group. Analysis of the data by phenotype demonstrated no significant (P>0.05) difference in the initial lactic acid concentrations between the DM phenotypes nor between the DM phenotypes and the normal-muscled non-carrier SY breed group. The initial lactic acid concentrations observed in the present study were slightly lower than those reported by Holmes *et al.* (1972a, 1973) for normal Hereford cattle and cattle with Inherited Muscular Hypertrophy (MH) sampled by jugular venipuncture. Strath *et al.* (1980) observed lower values when samples were obtained by jugular catheter from 18-month old half-sibling MH and normal-muscled bulls.

The phenotype differences observed in this study for initial plasma glucose and blood lactic acid concentrations are not in agreement with results reported by Holmes *et al.* (1972a, 1973). These workers reported significantly higher mean plasma glucose and blood lactic acid concentrations in MH Angus as compared to normal-muscled Hereford cattle, sampled by jugular venipuncture. Despite the possible confounding effect of breed in their study, Holmes and co-workers suggested that these differences may reflect the greater degree of excitability of MH cattle as compared to normal cattle and that the greater excitability of MH cattle may be a consequence of their higher blood lactic acid. However, Strath *et al.*(1980) observed no significant influence of mild temperature or nutritional stressors nor of animal type on plasma glucose and blood lactic acid concentrations for extreme-muscled DM cattle and carriers of the "double-muscling" trait, sampled by jugular catheter. In another study in which blood samples were obtained by jugular catheter, plasma glucose concentrations before and after a mild nutritional stress in homozygous and heterozygous MH Angus bulls were reported to be lower than in normal-muscled Angus bulls (Holmes and Robinson 1970). Holmes and Robinson (1970) therefore suggested that since MH cattle have decreased energy reserves stored as fat and a reduced ability to mobilize them, these animal types during times of increased energy demand must utilize gluconeogenic amino acids and volatile fatty acids to satisfy their energy demand. This may lead to a reduction in glucose precursors (Holmes and Robinson 1970) and a lowering of blood glucose. Blood lactic acid concentrations during the action of mild stressors have been variable (Holmes *et al.* 1973; Strath *et al.* 1980). However, during the action of more severe stressors such as prolonged exercise and epinephrine injection, blood lactic acid concentration has been observed to increase at a more rapid rate in MH as compared to normal cattle (Holmes and Robinson 1970; Holmes *et al.* 1972c, 1973). Holmes and co-workers (Holmes and Ashmore 1972; Holmes *et al.* 1973) suggest that this rapid increase in blood lactic acid in MH cattle exposed to exercise, metabolic or possibly blood sampling stressors is due to their increased muscle glycolytic capacity and reduced size of heart, liver, kidney (Oliver and Cartwright 1968) and oxidative muscle fibers which utilize lactic acid. Thus, the type, severity and length of stressors imposed on MH cattle can influence the proportion of glycolytic versus oxidative metabolism of their muscle. Since blood sampling by venipuncture imposes varying degrees and types of stress on the animal, variation in blood parameters affected by stressors may be expected. Further, concentrations of blood metabolites are of limited value unless one knows their flux.

The least squares mean initial hematocrit value of 38.9±0.6% observed for SY cattle was significantly ($P<0.05$)

higher than the value of 36.8±0.06% observed for cattle of the DM breed group. Analysis of the data by phenotype showed that initial hematocrit values between phenotypically extreme-muscled and phenotypically normal- to moderate-muscled cattle of the DM breed group were not significantly (P>0.05) different (Table VI.1). Other studies (for example, Appendix 9) carried out by the present author in which blood samples were collected by jugular venipuncture have shown that differences in hematocrit values between DM phenotypes and between DM phenotypes and normal-muscled SY cattle were variable. This seems to indicate that the breed group difference in hematocrit values observed in the present study was more related to the stressors imposed during sampling. This suggestion seems more credible since cattle of the DM breed group have been exposed to much more handling and confinement stressors than cattle of the SY breed group. Thus the higher plasma glucose and blood lactic acid concentrations observed for SY cattle in the present study may also be due to the greater effect of stressors on unfamilar animals. The correlation coefficients for hematocrit with plasma glucose concentration and blood lactic acid concentration with plasma glucose concentration seem to be in agreement (Table VI.2). In a study in which hematocrit values were not confounded by stress, lower values for both extreme-muscled DM cattle and carriers as compared to normal cattle have been reported (Monin and Boccard 1974). Ansay and Hanset

(1979) observed a similar result and stated that the lower hematocrit found in cattle exhibiting the "double-muscling" conformation may be related to the generalized hypotrophy of their non-muscular tissues (Oliver and Cartwright 1968; Monin and Boccard 1974), especially those affecting blood cell formation (bone and spleen).

As may be expected, the *in vitro* determinations of glucose consumption, lactic acid production and heat production by erythrocytes vary significantly depending on such experimental conditions as pH, temperature and glucose concentration (Murphy 1960; Garby and de Verdier 1964; Minakami and Yoshikawa 1966; Rapoport 1968; Travis *et al.* 1971; Levin 1973; Monti and Wadso 1976b). Within physiological limits, pH is positively correlated with erythrocyte heat production, glucose consumption and lactate production (Murphy 1960; Garby and de Verdier 1964; Minakami and Yoshikawa 1966; Rapoport 1968; Monti and Wadso 1976b). In samples monitored for pH during the present 4 h, *in vitro*, incubation study, pH dropped by 0.05 to 0.1 units. Rapoport (1968) states that in order to keep pH constant during the *in vitro* incubation of erythrocytes, buffers of high capacity such as 0.2 M-tris-HCl may be used. In the present study the concentration of tris buffer in the incubated whole blood was 1.53 mM. Thus, the glucose consumption and lactate production values determined in this study may lack quantitative significance, though, they do serve for comparisons. Further, the differences in erythrocyte glucose consumption and lactate production values observed between cattle of the DM breed group and normal cattle (Table VI.1) are probably under estimated because of this pH effect on erythrocyte glycolysis.

Throughout the present incubation study, the temperature of the medium was maintained at 37°C. Plasma glucose concentrations at the beginning of the incubation period were variable (6-12 mM). Significant negative correlations were observed for initial plasma glucose concentration with glucose consumption and with lactic acid production by erythrocytes (Table VI.2). However, investigators have demonstrated no significant dependency of glucose consumption or lactic acid production by human erythrocytes when glucose concentration of the medium varied within the range of 4-50 mM (Garby and de Verdier 1964; Travis *et al.* 1971). Glucose concentrations less than 0.4-0.6 mM have been reported to influence glucose consumption by erythrocytes (Garby and de Verdier 1964: Rapoport 1968). Monti and Wadso (1976b) showed that heat production by erythroctyes was not significantly influenced when glucose concentrations were varied between 3 and 32 mM. A significant correlation ($r=0.57$, $P<0.001$) between glucose consumption and heat production by erythrocytes has been reported (Monti and Wadso 1976a). In the present incubation study, heparin was used as the anticoagulant. This compound has not been shown to significantly influence the metabolism of glucose by erythrocytes (Guest *et al.* 1953; Levin 1973).

Other factors such as hemoglobin concentration (Monti 1977), external concentrations of Mg and Ca and extracellular Na and K within physiological limits were demonstrated to have no significant influence on glucose consumption by human erythrocytes (Garby and de Verdier 1964). Further, no significant correlations were observed in the present study between hematocrit and glucose consumption or lactic acid production rates by blood cells or between initial blood lactic acid concentration and lactic acid production rates by blood cells (Table VI.2).

The least squares mean glucose consumption by blood cells of 0.56±0.03 mmole/L packed cells/h observed for all cattle of the SY breed group was significantly (P<0.01) lower than the mean of 0.73±0.03 mmole/L packed cells/h observed for all cattle of the DM breed group (Fig. VI.1). When the data were analyzed by phenotype, the erythrocyte glucose consumption in phenotypically extreme-muscled DM cattle and phenotypically normal- to moderate-muscled cattle of the DM breed group were not significantly (P>0.05) different (Table VI.1). The mean values observed for glucose consumption rates by blood cells in the present study are in agreement with the rates of 0.58, 0.75±0.04 and 0.65±0.09 mmole glucose/L erythrocytes/h reported by Laris (1958), Love *et al.* (1974) and Love *et al.* (1977) for cattle of various ages and sexes and with the rates of 0.69±0.19 and 0.63±0.004 mmole glucose/L erythrocytes/h reported by Leng and Annison (1962) and Sutton (1976) for sheep of various ages and sexes.

The least squares mean lactic acid production by blood cells of 0.97±0.06 mmole/L packed cells/h for all SY cattle was significantly ($P<0.01$) lower than the mean of 1.38±0.05 mmole/L packed cells/h observed for all cattle of the DM breed group. The lactic acid production curve, over the 4 h incubation period, clearly illustrates this breed group difference (Fig. VI.2). No significant ($P>0.05$) difference was observed in lactic acid production by blood cells between phenotypically extreme-muscled and phenotypically normal- to moderate-muscled cattle of the DM breed group (Table VI.1). The mean values for lactic acid production rates by blood cells observed in the present study are similar to the rates of 1.62±0.29 and 1.18±0.01 mmole lactic acid/L erythrocytes/h reported by Leng and Annison (1962) and Sutton (1976) for sheep.

Both glucose consumption and lactic acid production by blood cells were linear over the 4 h incubation period (Fig. VI.1 and VI.2), with an average of the r^2 values for all animals in the present study of 0.96±0.005 and 0.96±0.007, respectively. This linear relationship for glucose consumption and lactic acid production by erythrocytes from various mammalian species over a 4 h, *in vitro*, incubation (37°C) period has been observed by other workers (Laris 1958; Garby and de Verdier 1964; Agar and O'Shea 1975; Sutton 1976).

The least squares mean lactic acid production to glucose consumption molar ratio for blood cells of 1.76±0.10 for all SY cattle was not significantly ($P>0.05$) different from the mean of 1.92±0.08 observed for all cattle of the DM breed group. No significant ($P>0.05$) difference was observed in this ratio between DM phenotypes or between the DM breed group and the normal-muscled non-carrier SY cattle (Table VI.1). The mean lactic acid to glucose molar ratios observed for blood cells in the present study are in agreement with those of 1.93 and 1.86 observed for sheep erythrocytes by Agar and O'Shea (1975) and Sutton (1976). Approximately 94% of the glucose carbons which were consumed by the blood cells, in the present study, appeared as lactic acid. This was calculated on the basis that, anaerobically, two moles of lactic acid are produced from one mole of glucose. This is in agreement with other workers who reported that 90-95% of glucose metabolized by erythrocytes in various mammalian species including cattle and sheep flows through the EMP, with 5-10% of the glucose carbons being lost to other pathways such as the pentose phosphate pathway (Yunis and Yashmineh 1969; Kaneko 1974; Love *et al.* 1974; Agar and O'Shea 1975; Harvey and Kaneko 1976). Thus, the lactic acid to glucose molar ratios and the lactic acid production rates observed for cattle blood cells in the present study are in agreement with values reported in the literature for cattle.

The only significant effect of sex observed in the present study was on lactic acid production by blood cells.

Values for male and female animals were 1.39±0.06 and 1.14±0.04 mmole lactic acid/L packed cells/h ($P<0.01$), respectively. Animal age was not observed to significantly ($P>0.05$) influence any of the measured parameters when data were analyzed by breed group or phenotype. No biologically significant interactions effects were observed.

In the present study leukocytes were not removed from the blood samples prior to incubation. This raises the possibility that the differences observed in glucose consumption and lactic acid production by phenotype were due to differences in leukocyte counts. The rate of glycolysis in leukocytes is 200 to 900 times greater than that in erythrocytes and contribute significantly to the total glycolysis in blood (Guest *et al.* 1953) even though erythrocytes out number leukocytes by 700-900 to 1 in cattle (Appendix 9). In the data presented in Appendix 9, no significant differences were observed by phenotype in erythrocyte, leukocyte or differential leukocyte counts nor in any of the other hematological parameters presented. Thus, the results of the present study demonstrated that the rates of glycolysis in blood cells from extreme-muscled DM cattle and carriers of the "double-muscling" trait were significantly increased as compared to normal-muscled non-carrier cattle of the SY breed group. Kolataj and associates (Kolataj *et al.* 1979a, b; Konecka *et al.* 1979) have measured the activity of certain plasma enzymes in normal, heterozygous and double muscled cattle and suggested that double muscled cattle have an increased rate of glycolysis. Kolataj *et al.* (1979a) also speculated that double muscled cattle have an increased metabolic rate. This speculation has since been confirmed by Strath *et al.* (1981).

In a previous study (Chapter II), it was reported that extreme-muscled DM cattle demonstrate a significantly increased susceptibility of their erythrocytes to osmotic lysis as compared to carriers of the "double-muscling" trait and to normal-muscled non-carrier cattle of the SY breed group (Basarab *et al.* 1980). The results of the present study suggest that the erythrocytes from phenotypically extreme-muscled DM cattle have increased their rate of glycolysis, thus increasing ATP, NADH and NADPH production, in order to maintain the integrity of an abnormal, possibly more fragile or "leaky" membrane. Other evidence has been reported to support an increased glucose metabolism in erythrocytes from extreme-muscled DM cattle. For example, Strath *et al.* (1980) reported increased serum triiodothyronine (T_3) concentrations in phenotypically extreme-muscled DM cattle as compared to carriers of the "double-muscling" trait. The present author had previously observed (Chapter III) an increased serum T_3 concentration and a trend toward increased serum thyroxine concentrations in phenotypically extreme-muscled DM cattle as compared to carriers of the "double-muscling" trait and to normal-muscled non-carrier cattle of the SY breed group. It may be suggested that the increased serum T_3 concentrations associated with the extreme-muscled cattle in the DM breed group may be involved in the mediation of their increased erythrocyte glucose metabolism, since other workers (Necheles and Beutler 1959; Snyder *et al.* 1971; Monti and Wadso 1976a) have demonstrated that thyroid hormones significantly increase glucose consumption and heat production by erythrocytes. However, Monti and Wadso (1976a) have also discussed the possibility of thyroid hormones altering glucose metabolism of erythrocytes by increasing the activity of the pentose phosphate pathway. In the present study no significant difference was observed in the lactic acid to glucose molar ratios by phenotype (Table VI.1), thus indicating no significant difference in the way in which erythrocytes from each phenotype metabolized glucose.

An interesting aspect of the present study and of others carried out by the present author was the significantly increased rate of erythrocyte glycolysis observed for carriers of "double-muscling", without a corresponding significant increase in erythrocyte osmotic fragility (Chapter II) and serum T_3 concentration (Chapter III), as compared to normal-muscled non-carrier cattle of the SY breed group. This apparent inconsistency in the relationship carriers have, in many parameters studied, to extreme-muscled DM cattle and to normal cattle has been observed by others (Oliver and Cartwright 1968; Holmes and

Robinson 1970; West 1974). Depending on the parameter under study, carriers can be either more like the extreme-muscled DM animals, normal cattle or be intermediate. Thus, as suggested by other workers (Oliver and Cartwright 1968; Kieffer *et al.* 1972a, b; Rollins *et al.* 1972; Kieffer and Cartwright 1980; Chapter I) the expression of the "double-muscling" trait and its manifestations in the heterozygote has incomplete penetrance. The phenotypic expression of the "double-muscling" trait and the number and degree of traits associated with its expression will therefore depend on the proportion of a buffering genotype present (Chapter I). It may therefore be suggested that the increased rate of erythrocyte glycolysis observed in the present study for carriers may have been sufficient to maintain their erythrocyte osmotic fragility within the normal range. In the case of the extreme-muscled DM cattle, the increased glucose metabolism by their erythrocytes may be preventing severe physiological damage, however may not be sufficient to maintain their erythrocyte osmotic fragility within the normal range.

In conclusion, the results of the present study have demonstrated that all carriers of the "double-muscling" trait, regardless of phenotypic expression of the trait, have an increased rate of erythrocyte glycolysis as compared to normal-muscled non-carrier cattle. This increased rate of erythrocyte glycolysis may be in response to the maintenance of the integrity of an abnormal, possibly more fragile or "leaky" erythrocyte membrane. What mediates this increased erythrocyte glucose metabolism is unknown, though thyroid hormones have been implicated. Further, the extrapolation of an abnormal erythrocyte membrane to a more generalized membrane defect as suggested by King *et al*. (1976a) and Basarab *et al*. (1980), has important implications to the overall energy metabolism and physiology of cattle which are carriers of the "double-muscling" trait.

Table VI.1 Least squares means ± SEM of initial glucose and lactate concentration, initial PCV, glucose consumption and lactate production and lactate:glucose molar ratio for whole blood by phenotype.

Breed Group	SY	DM		
Phenotype[1]	Normal	Normal to Moderate	Extreme	Sig[2]
Animals[3]	20	15	10	
Initial Glucose (mmole/L plasma)[4]	9.26±0.18*a*	7.58±0.21*b*	7.68±0.25*b*	**
Initial Lactic Acid (mmole/L blood)	2.68±0.49	1.47±0.58	2.18±0.70	
Initial Hematocrit (%)	38.9 ±0.6 *a*	36.6 ±0.7 *b*	35.8 ±0.8 *b*	*
Glucose Consumption (mmole/L packed cells/h)	0.56±0.03*a*	0.71±0.04*b*	0.78±0.04*b*	**
Lactate Production (mmole/L packed cells/h)	0.96±0.05*a*	1.40±0.06*b*	1.42±0.08*b*	**
mmole lactate/L packed cells/h / mmole glucose/L packed cells/h	1.76±0.10	1.98±0.12	1.86±0.14	

[1] Based on a subjective score for muscling.

[2] *ab*Means in the same row with different italicized letters indicate a significant phenotype difference: *P<0.05; **P<0.01.

[3] Number of animals per phenotype.

[4] Initial glucose contains 4.47 and 4.30 mmole added glucose/L plasma for the SY and DM breed groups, respectively.

Table VI.2 Simple correlations among experimental variables across all treatment groups.

Variables	Initial Plasma Glucose	Initial Blood Lactic Acid	Initial Hematocrit	Glucose Consumption Rate	Lactic Acid Production Rate	Lactic Acid to Glucose Molar Ratio
Initial Plasma Glucose	1	......	......	......	......	......
Initial Blood Lactic Acid	0.428**	1	......	......	......	......
Initial Hematocrit	0.506**	0.096	1	......	......	......
Glucose Consumption Rate	-0.293*	-0.295*	-0.105	1	......	......
Lactic Acid Production Rate	-0.676**	-0.196	-0.229	0.657**	1	......
Lactic Acid to Glucose Molar Ratio	-0.472**	0.129	-0.143	-0.225	0.571**	1

*P<0.05; **P<0.01.

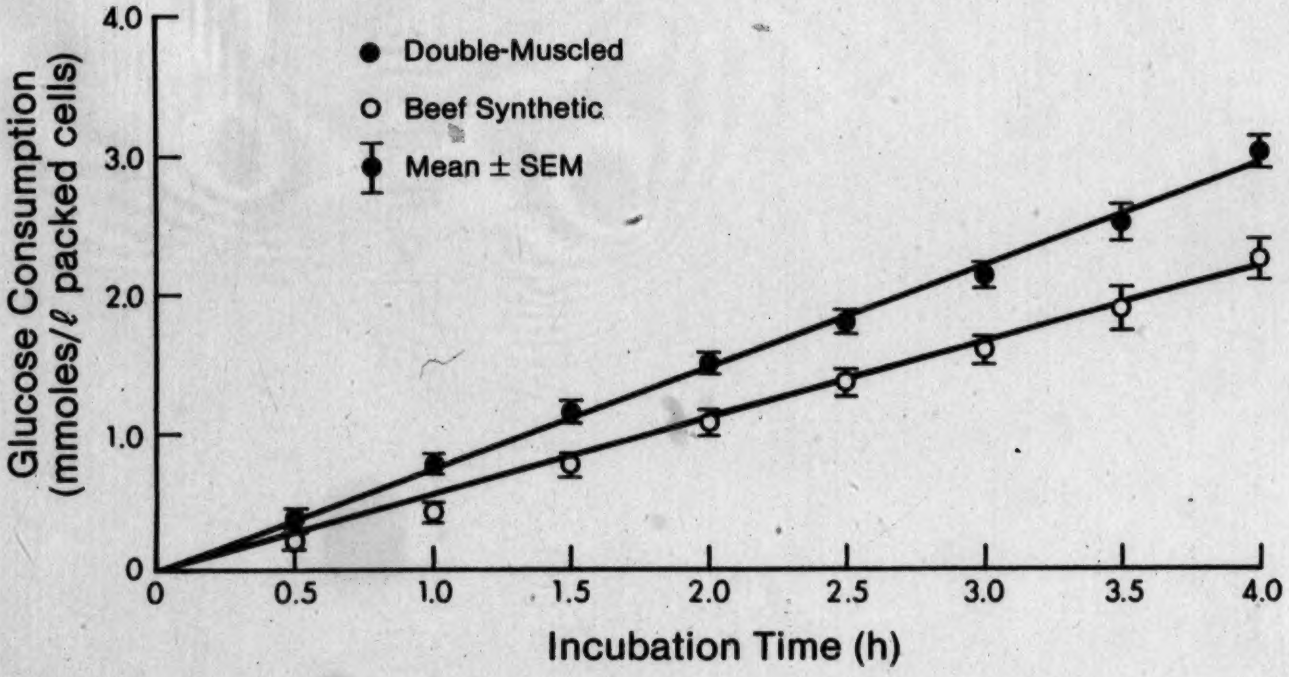

Figure VI.1 Mean blood cell glucose consumption by breed group during a 4 h, in vitro, incubation (37°C) period.

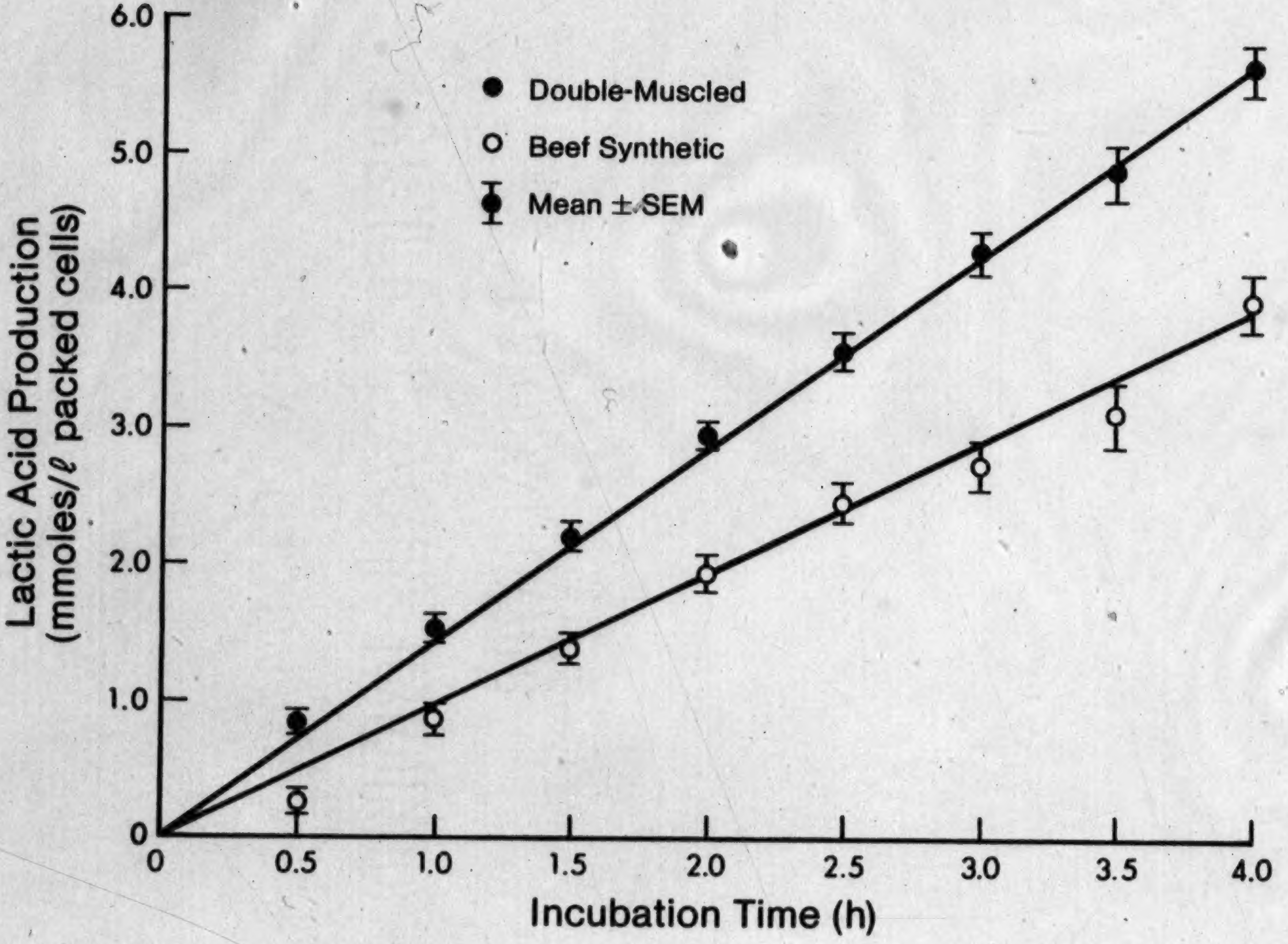

Figure VI.2 Mean blood cell lactic acid production by breed group during a 4 h, in vitro, incubation (37°C) period.

GENERAL SUMMARY AND CONCLUSIONS

The objectives of this work were to examine the mode of inheritance of the "double-muscling" trait in cattle, to verify the occurrence of a membrane defect in these animals and to examine its nature and manifestations.

Mode of inheritance

The investigation reported herein on the mode of inheritance of the "double-muscling" trait in cattle supports the evidence which suggests that this trait is under the control of a single recessive gene pair with variable expression in the heterozygote mediated by modifying gene-products. The possibility that the trait is quantitative and under the influence of many genes is still present.

Membrane defect, nature and manifestations

Phenotypically extreme double-muscled (DM) cattle were demonstrated to have an increased susceptibility of their erythrocytes to osmotic lysis as compared to carriers of the "double-muscling" trait and to normal cattle. This finding was thought to be indicative of a generalized membrane defect in double-muscled cattle. Examination of the lipid composition of erythrocyte membranes from extreme-muscled DM cattle revealed alterations in the fatty acid composition of their erythrocyte membrane phospholipids as compared to normal-muscled carriers. These alterations in fatty acid composition may, to some degree, contribute to the increased erythrocyte osmotic fragility observed in extreme-muscled DM cattle. The altered fatty acid composition observed in the erythrocyte membranes from these animal types would undoubtedly alter the fluidity and permeability and thus the functional state of this membrane system.

From an evolutionary point of view, membrane systems have evolved to a complexity where sudden changes in their functional integrity, brought about either as a direct or indirect result of a genetic disorder, have a high probability of being deleterious. In order to maintain homeostasis, and thus survival of the organism, the cell must bring into play certain counter-balancing mechanisms. The increased production of ATP, NADH and NADPH, which play a major role in maintaining membrane function, would therefore be of consequence. Thus, the observed increased rate of glucose consumption and lactate production by erythrocytes from extreme-muscled DM cattle and carriers as compared to normal cattle is suggestive of an increased erythrocyte glycolytic rate in order to maintain an altered, possibly "leaky", membrane.

The above observations, their extrapolation to other membrane systems, the observed increased triiodothyronine concentration in extreme-muscled as compared to normal cattle and the decreased concentration of triacylglycerides in plasma and plasma lipoproteins from extreme-muscled DM cattle as compared to normal-muscled carriers all point to an increased demand for energy in phenotypically extreme-muscled DM cattle.

Biochemical abnormality responsible

The studies reported herein as well as observations reported by others seem to point to two alternative explanations as to the primary biochemical lesion responsible for the Double Muscled Syndrome in cattle. The first suggests that the gene(s) responsible produces a gene product(s) which mediates its expression by altering the structural integrity of cell membranes. This results in a proposed disruption of neuromuscular contact which in turn results in hypertrophy and hyperplasia of myofibers, alters cellular metabolism and hence muscle fiber type. All other physical, physiological, histological and biochemical characteristics associated with this syndrome are therefore proposed to be the result of the initial alterations in cell membranes. The second hypothesis suggests that the gene(s) responsible for "double-muscling" produces a gene product(s) which alters some aspect of endocrine or neuroendocrine functionality. This in turn alters cellular differentiation, growth and metabolism. Both explanations appear to be equally plausible. Thus at the molecular level we run into an old puzzle about the chicken or the egg. Which came first, alterations in cell membrane functionality or alterations in endocrine or neuroendocrine status? The answer to this question and that of others lies in the further experimental investigation of the Double Muscled Syndrome in cattle.

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APPENDICES

Appendix 1. Determination of correction factors for plasma lipids and phospholipid classes on the Iatroscan TH-10 Mark-II Analyzer TLC/FID system.

Appendix 2. Percent and average percent lipid class composition ± SEM of lipid standard solution for the determination of correction factors (CF) for rod and run variation and response of cholesterol esters, triacylglycerides, free fatty acids, cholesterol and phospholipids on the Iatroscan TH-10 Mark-II Analyzer.

Appendix 3. Percent and average percent phospholipid class composition ± SEM of lipid standard solution for the determination of correction factors (CF) for rod and run variation and response of phosphatidylcholine and sphingomyelin on the Iatroscan TH-10 Mark-II Analyzer.

Appendix 4. An example of a chromatogram for erythrocyte membrane lipids in cattle.

Appendix 5. A typical chromatogram of lipid standards used for the determination of lipid correction factors and identification of plasma lipids.

Appendix 6. A typical chromatogram of phospholipid standards used for the determination of correction factors for plasma phospholipids.

Appendix 7. An example of a chromatogram for plasma lipid classes in cattle.

Appendix 8. An example of a chromatogram for plasma phospholipid classes in cattle.

Appendix 9. Least squares means of some hematology parameters in normal-muscled cattle of a Beef Synthetic breed group and normal- to moderate-muscled and extreme-muscled cattle of a "Double-Muscled" breed group.

Appendix 1. Determination of correction factors for plasma lipids and phospholipid classes on the Iatroscan TH-10 Mark-II Analyzer TLC/FID system.

Lipid Standard:

1. A neutral lipid standard (No. 178-4, 100 mg) containing 25% cholesterol, 24.9% cholesteryl oleate, 25.1% oleic acid and 25% triolein obtained from Sigma Chemical Co., St. Louis, Mo. 63178, U.S.A., was dissolved in pentane.

2. An aliquot of this neutral lipid standard was transferred to a pre-weighed scintillation vial. This lipid solution was evaporated to dryness under nitrogen and weighed. There was a total of 16.1 mg of neutral lipid in the vial.

3. To the vial containing the neutral lipid standards was added 500 uL of a solution of chloroform:methanol (2:1, v/v) containing 4 mg/mL phosphatidylcholine and 1 mg/mL sphingomyelin. Chloroform:methanol (1.5 mL, 2:1, v/v) was added to the vial making the final working lipid standard concentration equal to 9.3 mg/mL. The final composition of the working lipid standard solution was 21.64% cholesterol, 21.55% cholesteryl oleate, 21.73% oleic acid, 21.64% triolein and 13.44% phospholipid (80% phosphatidylcholine and 20% sphingomyelin).

All solvents were reagent grade and were glass distilled before use.

Procedure:

1. A 5 uL microsyringe was used to spot approximately 1 uL of the lipid standard solution per chromarod S-II of a set of 10 rods contained in a metal frame rod holder.

2. The frame containing the spotted rods was then placed into a filter paper lined glass tank and developed for 45 min in a solvent mixture of methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15).

3. The frame was removed from the tank and dried in a forced air oven at 60°C for 3 min.

4. This frame was then transferred to the scanning stage of the Iatroscan TH-10 Mark-II Analyzer (Iatron Laboratories Inc., Tokyo, Japan, world-wide factory representatives and marketing consultants: Newman-Howells Associates Ltd., Winchester, United

Kingdom) and each rod was scanned completely by passing through the FID head.

5. The FID was operated with a hydrogen gas flow of 160 mL/min from a pressure of 0.75 kg/cm^2 and compressed air at a flow of 2000 mL/min. A 30-tooth gear was used for the automatic scanning of the ten rods. The recorder chart speed was 20 cm/min and sensitivity was set at 0.1 volts.

6. A Houston Model B5217 2-pen flat-bed chart recorder connected to the Iatroscan TH-10 analyzer was used to record the lipid peaks and their integrations (Appendix 3 and 4).

7. A total of eight runs of the lipid standard solution per set of ten rods was carried out for the determination of the correction factors for lipid classes (Appendix 2).

8. The integration number for each lipid/rod/run were determined. Calculation of the percent composition of each lipid in the lipid standard solution/rod/run were determined by the following formula:

$$\frac{\text{Integration no. of lipid(a) on rod(b) for run(c)} \times 100}{\text{Total integration no. of all lipids on rod(b) for run(c)}},$$

where

a = cholesterol esters (CE), triacylglycerides (TG), free fatty acids (FAA), cholesterol (C) or phospholipid (PL),
b = rod numbers 1 to 10 and
c = run numbers 1 to 8.

9. The percent composition of each lipid/rod/run (Appendix 2) were then analyzed using the one-way analyses of variance (Steel and Torrie 1980).

10. Correction factors (Appendix 2) for the response of each lipid on each rod was determined as follows:

$$\frac{\text{Actual \% of lipid(a) in standard lipid solution}}{\text{Observed \% of lipid(a) on rod(b) for run(c)}},$$

where

a = CE, TG, FFA,C or PL,
b = rod numbers 1 to 10 and
c = run numbers 1 to 8.

11. Calculation of percent composition of lipid classes in

samples were determined as follows:

$$\frac{\text{Sample integration no. of lipid(a) on rod(b) for run(c)}}{\text{Total sample integrations of lipids on rod(b) for run(c)}}$$

x 100 x average CF for lipid(a) on rod(b), where

a = CE, TG, FFA, C or PL,
b = rod numbers 1 to 10 and
c = run numbers 1 to 8.

12. The working lipid standard solution used for the determination of lipid class correction factors was also used for phospholipid class correction factor determination.

13. Approximately 1 uL of the lipid standard solution was spotted on each rod of a set of 10 rods contained in a metal frame rod holder.

14. The frame containing the spotted rods was then placed into a developing tank and developed for 45 min in a solvent mixture of methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15). The rods were dried, scanned down to the phospholipid band, re-developed for 1 h in chloroform:methanol:water (80:30:3.5) and then scanned completely to obtain standard phospholipid class peaks and their integrations (Appendix 3).

15. Correction factors for the phospholipid classes (Appendix 3) were then determined the same as those for lipid classes. Correction factors for the minor phospholipid classes were assumed to equal 1.

Appendix 2. Percent and average percent lipid class composition ± SEM' of lipid standard solution for the determination of correction factors (CF) for rod and run variation and response of cholesterol esters, triacylglycerides, free fatty acids, cholesterol and phospholipids on the Iatroscan TH-10 Mark II Analyzer.

Rod No.	1	2	3	4	5	6	7	8	9	10
					Cholesterol esters					
Run No.										
1	16.43	17.64	18.66	15.79	16.77	15.45	19.13	16.07	18.04	17.70
2	16.43	18.53	20.08	17.18	16.03	16.20	19.43	18.73	18.79	16.92
3	17.03	17.76	19.86	17.80	18.02	16.09	18.51	16.47	17.45	16.93
4	17.21	18.25	19.51	17.63	17.20	15.97	18.92	18.46	17.82	15.89
5	18.08	18.18	19.54	17.93	16.85	16.49	18.59	17.18	18.51	17.92
6	17.72	17.16	18.93	17.39	18.60	15.97	18.35	17.05	18.56	17.12
7	16.94	17.86	18.96	17.60	16.28	16.62	18.90	17.73	18.97	18.33
8	18.22	18.36	20.05	17.94	17.76	15.96	19.07	18.12	18.82	18.12
Ave	17.26	17.97	19.45	17.41	17.19	16.09	18.86	17.48	18.37	17.37
SEM'	±0.24	±0.16	±0.19	±0.25	±0.31	±0.13	±0.13	±0.34	±0.19	±0.28
CF	1.25	1.20	1.11	1.24	1.25	1.34	1.14	1.23	1.17	1.24
Overall SEM'=0.23										
					Triacylglycerides					
Run No.										
1	17.36	17.83	17.51	16.45	18.92	17.63	18.79	17.82	18.57	17.03
2	17.36	17.97	17.89	16.70	16.52	17.37	18.95	18.34	19.31	17.05
3	16.53	16.39	17.90	16.98	16.35	16.23	18.10	16.67	18.24	17.29
4	17.54	18.04	17.96	16.79	18.10	16.81	18.80	18.12	18.29	17.58
5	17.98	17.73	17.35	17.37	18.35	17.28	18.72	17.18	18.75	17.92
6	18.14	17.01	17.33	16.69	17.67	16.95	18.24	17.19	18.70	17.91
7	17.92	18.02	17.98	17.02	17.92	17.41	19.01	17.93	19.01	18.02
8	17.81	17.95	18.70	17.44	18.55	16.99	19.01	18.12	19.13	18.12
Ave	17.58	17.62	17.83	16.93	17.80	17.08	18.70	17.67	18.75	17.61
SEM'	±0.18	±0.21	±0.16	±0.12	±0.33	±0.16	±0.12	±0.21	±0.14	±0.16
CF	1.23	1.23	1.21	1.28	1.22	1.27	1.16	1.22	1.15	1.23
Overall SEM'=0.19										

Continued-

Rod No.	1	2	3	4	5	6	7	8	9	10
					Free Fatty Acids					
Run No.										
1	21.13	22.25	20.97	21.93	20.43	22.55	21.46	23.72	22.41	21.43
2	21.13	22.67	20.78	21.62	19.38	22.61	22.13	22.60	22.82	21.91
3	23.23	22.47	20.82	19.89	20.88	23.18	21.87	23.31	22.65	21.48
4	23.20	23.09	20.86	20.76	20.57	22.03	21.82	22.15	21.07	22.65
5	22.32	22.20	20.63	20.14	21.15	22.66	22.02	22.57	21.86	21.85
6	23.02	23.13	19.98	19.71	20.21	23.54	21.71	23.64	22.21	21.34
7	22.33	22.10	20.51	20.17	20.00	22.15	20.40	22.68	21.32	21.48
8	21.81	21.98	20.57	20.27	20.69	23.17	21.08	23.29	22.00	21.20
Ave	22.27	22.49	20.64	20.56	20.41	22.74	21.56	23.00	22.04	21.67
SEM'	±0.30	±0.16	±0.11	±0.29	±0.20	±0.19	±0.20	±0.20	±0.22	±0.16
CF	0.98	0.97	1.05	1.06	1.06	0.96	1.01	0.94	0.99	1.00
Overall SEM'=0.21										
					Cholesterol					
Run No.										
1	27.23	27.48	27.46	29.77	29.21	28.92	27.80	27.77	26.77	29.31
2	27.23	26.49	26.45	30.58	33.11	28.46	26.86	25.85	26.55	27.71
3	27.36	26.86	26.51	29.89	28.78	28.33	27.01	27.20	26.91	28.70
4	27.36	27.74	27.22	29.61	28.85	27.97	27.48	25.69	25.41	29.47
5	26.06	26.75	26.95	29.13	28.67	28.50	26.91	26.33	26.37	28.55
6	26.91	27.39	27.41	28.77	27.83	28.72	27.60	27.49	26.23	28.40
7	26.95	27.55	27.06	29.24	29.13	28.19	27.36	26.91	26.30	28.11
8	26.23	26.51	26.53	29.01	27.71	28.35	26.37	26.61	25.71	27.97
Ave	26.92	27.10	26.95	29.50	29.16	28.43	27.17	26.73	26.28	28.52
SEM'	±0.18	±0.18	±0.14	±0.21	±0.60	±0.10	±0.17	±0.26	±0.18	±0.22
CF	0.80	0.80	0.80	0.73	0.74	0.76	0.80	0.81	0.82	0.76
Overall SEM'=0.26										

Continued-

Rod No.	1	2	3	4	5	6	7	8	9	10
	Phospholipids									
Run No.										
1	17.85	14.80	15.40	16.06	14.67	15.45	12.83	14.61	14.21	14.53
2	17.85	14.34	14.80	13.92	14.95	15.37	12.62	14.47	12.54	16.42
3	15.85	16.52	14.91	15.45	15.97	16.16	14.50	16.36	14.74	15.60
4	14.69	12.87	14.45	15.20	15.29	17.22	12.98	15.58	17.41	14.40
5	15.57	15.13	15.53	15.43	14.98	15.06	13.75	16.74	14.51	13.76
6	14.22	15.31	16.34	17.44	15.70	14.80	14.09	14.64	14.31	15.24
7	15.86	14.46	15.49	15.96	16.68	15.63	14.33	14.75	14.40	14.06
8	15.94	15.20	14.14	15.34	15.30	15.54	14.48	13.86	14.34	14.59
Ave	15.98	14.83	15.13	15.60	15.44	15.66	13.70	15.12	14.56	14.83
SEM[1]	±0.46	±0.37	±0.25	±0.35	±0.23	±0.26	±0.28	±0.35	±0.47	±0.31
CF	0.84	0.91	0.89	0.86	0.87	0.86	0.98	0.89	0.92	0.91
Overall SEM[1]=0.34										

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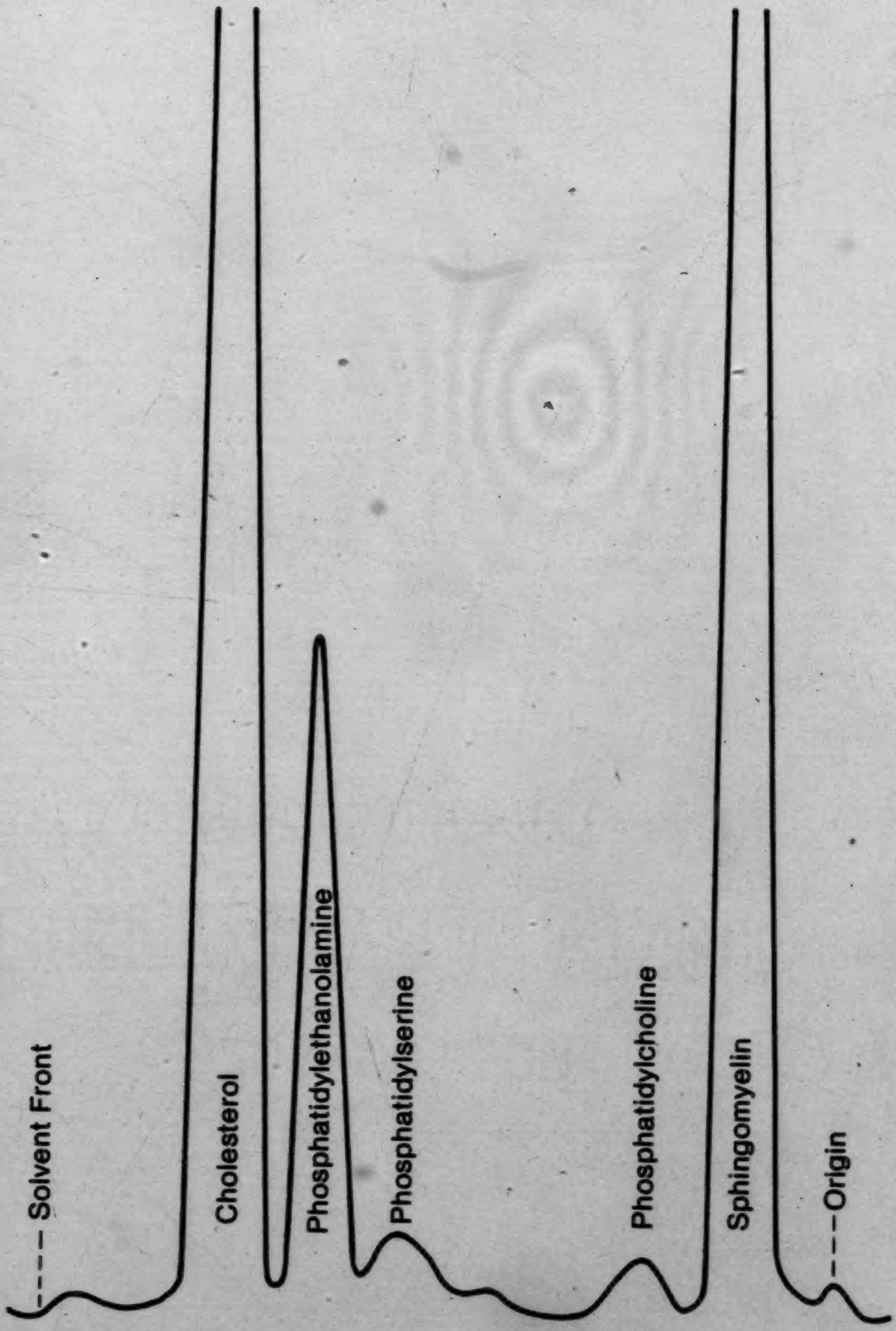
[1] Standard error of the mean.

Appendix 3. Percent and average percent phospholipid class composition ± SEM[1] of lipid standard solution for the determination of correction factors (CF) for rod and run variation and response of phosphatidylcholine and sphingomyelin on the Iatroscan TH-10 Mark II Analyzer.

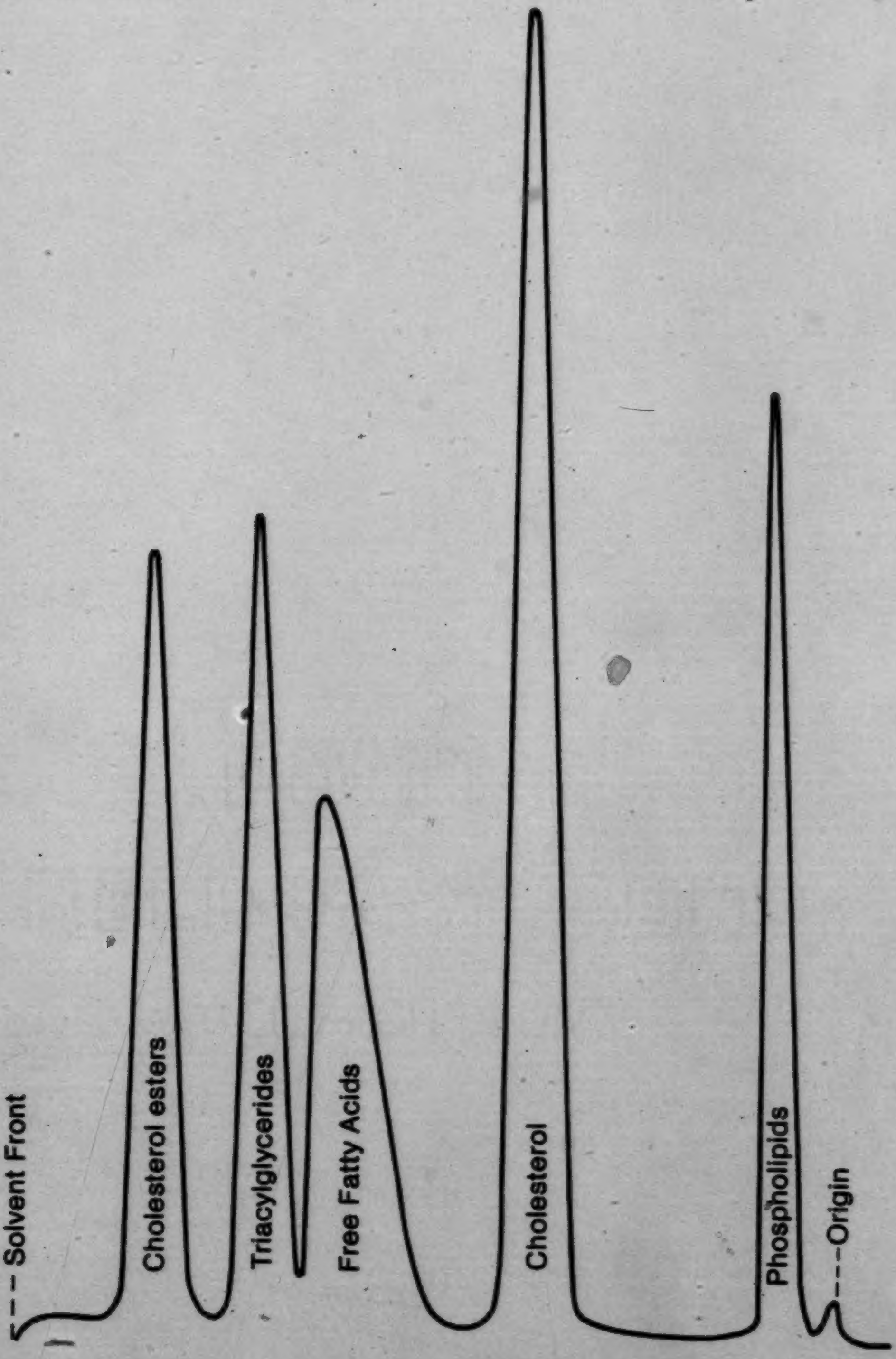
Rod No.	1	2	3	4	5	6	7	8	9	10
					Phosphatidylcholine					
Run No.										
1	80.36	79.33	79.90	81.21	81.02	81.21	80.53	80.38	81.26	82.94
2	81.38	79.18	77.46	80.14	80.28	79.76	80.45	80.88	81.61	82.36
3	81.83	80.03	79.60	80.65	81.55	81.15	81.37	80.34	81.41	80.00
4	80.69	79.43	79.68	80.07	78.42	79.27	79.69	79.07	79.96	81.69
Ave	81.07	79.49	79.16	80.52	80.32	80.35	80.51	80.17	81.06	81.75
SEM[1]	±0.33	±0.19	±0.57	±0.26	±0.68	±0.49	±0.34	±0.39	±0.37	±0.64
CF	0.99	1.01	1.01	0.99	1.00	1.00	0.99	1.00	0.99	0.98
Overall SEM[1]=0.45										
					Sphingomyelin					
Run No.										
1	19.64	20.67	20.10	18.79	18.98	18.79	19.47	19.62	18.74	17.06
2	18.62	20.82	22.54	19.86	19.72	20.24	19.55	19.12	18.39	17.64
3	18.17	19.97	20.40	19.35	18.45	18.85	18.63	19.66	18.59	20.00
4	19.31	20.57	20.32	19.93	21.58	20.73	20.31	20.93	20.04	18.31
Ave	18.93	20.51	20.84	19.48	19.68	19.65	19.49	19.83	18.94	18.25
SEM[1]	±0.33	±0.19	±0.57	±0.26	±0.68	±0.49	±0.34	±0.39	±0.37	±0.64
CF	1.06	0.98	0.96	1.03	1.02	1.02	1.03	1.01	1.06	1.10
Overall SEM[1]=0.45										

[1] Standard error of the mean.

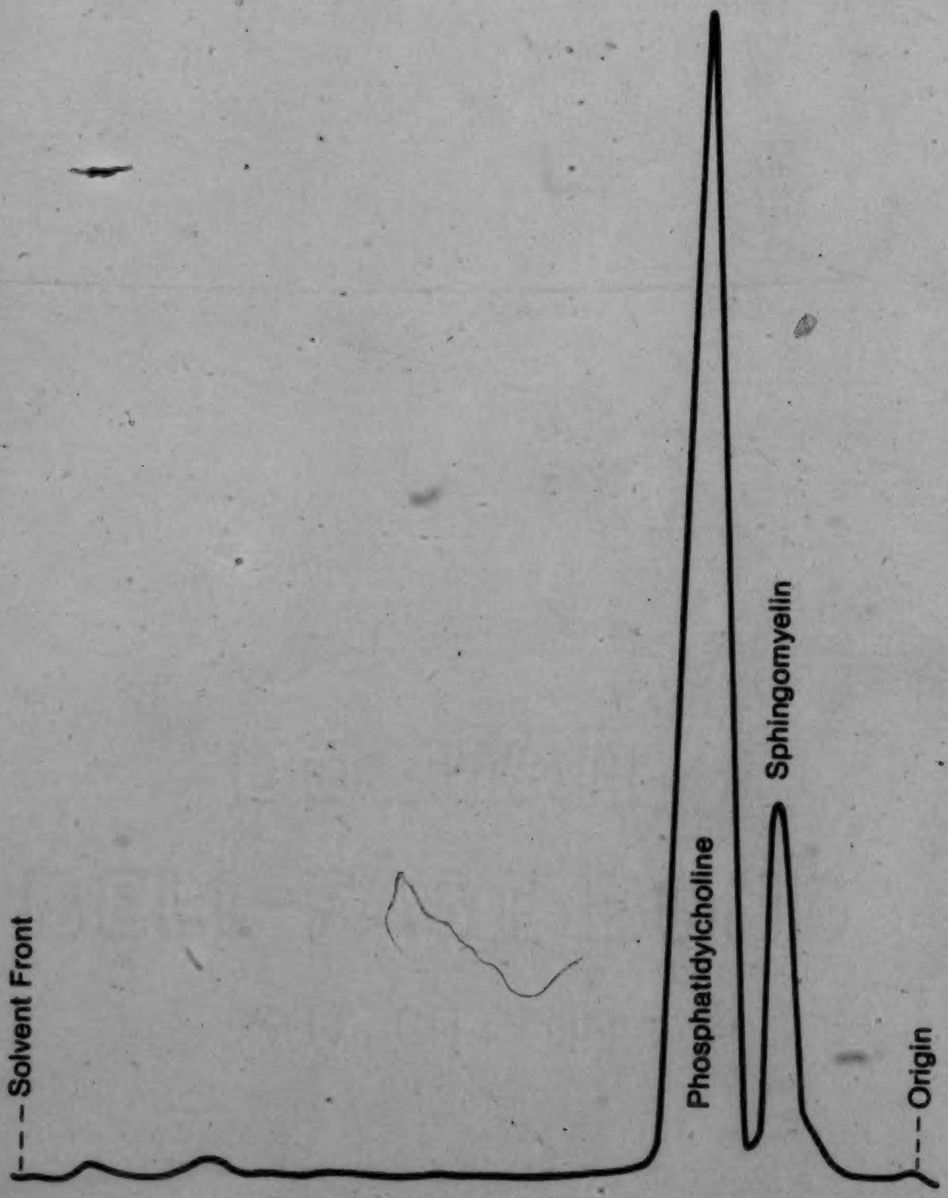
Appendix 4. An example of a chromatogram for erythrocyte membrane lipids in cattle. Rods were spotted with approximately 1 uL of erythrocyte membrane lipid extract and developed in chloroform:methanol:water (80:35:3.5) for 1 h.


Solvent Front
Cholesterol
Phosphatidylethanolamine
Phosphatidylserine
Phosphatidylcholine
Sphingomyelin
Origin

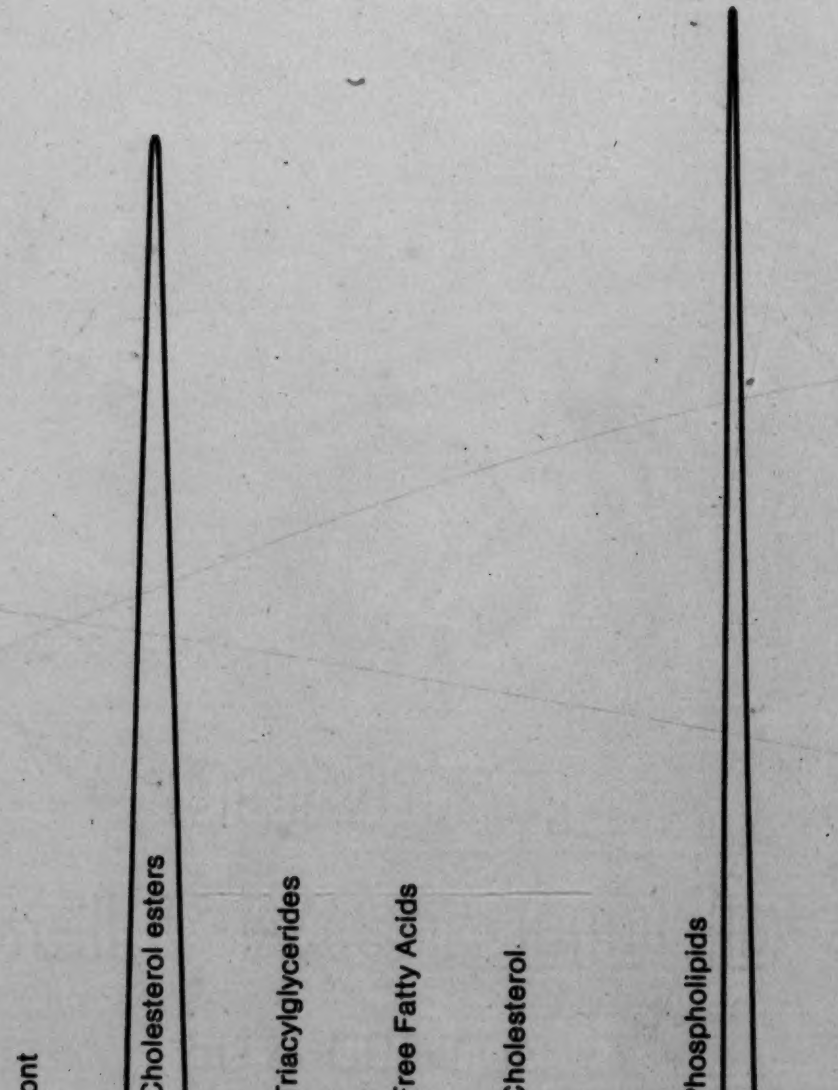
Appendix 5. A typical chromatogram of lipid standards used for the determination of lipid correction factors and identification of plasma lipids. Rods spotted with 1 uL/rod of lipid standards were developed in methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15) for 45 min.


Solvent Front
Cholesterol esters
Triacylglycerides
Free Fatty Acids
Cholesterol
Phospholipids
Origin

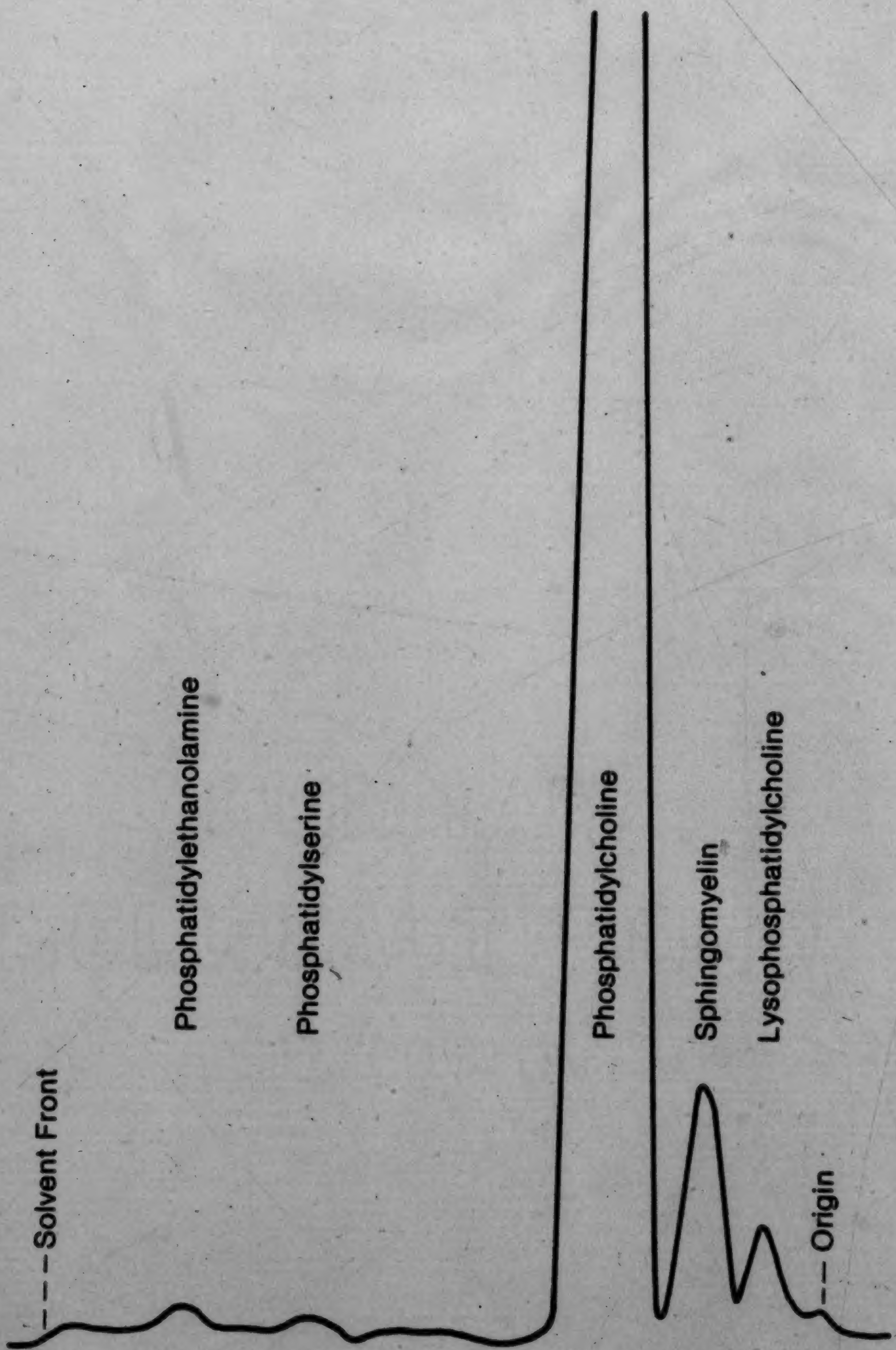
Appendix 6. A typical chromatogram of phospholipid standards used for the determination of correction factors for plasma phospholipids. Rods spotted with 1 uL lipid standard/rod were developed in methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15) for 45 min. The rods were scanned down to the phospholipid band, re-developed in chloroform:methanol:water (80:30:3.5) for 1 h and then scanned completely.


Solvent Front
Phosphatidylcholine
Sphingomyelin
Origin

Appendix 7. An example of a chromatogram for plasma lipid classes in cattle. Rods spotted with approximately 1 uL of plasma lipid extract/rod were developed in methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15) for 45 min.


Solvent Front
Cholesterol esters
Triacylglycerides
Free Fatty Acids
Cholesterol
Phospholipids
Origin

Appendix 8. An example of a chromatogram for plasma phospholipid classes in cattle. Rods spotted with approximately 1 uL of plasma lipid extract/rod were developed in methylene chloride:chloroform:acetic acid:methanol (98:8:0.4:0.15) for 45 min. The rods were scanned down to the phospholipid band, re-developed in chloroform:methanol:water (80:30:3.5) for 1 h and scanned completely.


Solvent Front
Phosphatidylethanolamine
Phosphatidylserine
Phosphatidylcholine
Sphingomyelin
Lysophosphatidylcholine
Origin

Appendix 9. Least squares means of some hematology parameters in normal-muscled cattle of a Beef Synthetic (SY) breed group and normal- to moderate-muscled and extreme-muscled cattle of a "Double-Muscled" (DM) breed group.[1]

Phenotype	N[2]	RBC 10^{12}/L	WBC 10^{9}/L	$\frac{RBC}{WBC}$	Hgb g/dL	HCT %	MCV 10^{-15}L	MCH pg	MCHC g/dL	POLYS %	LYMPHS %	MONOS %	EOS %
Normal SY	18	7.46	8.4	913	13.8	36.3	49.6	18.8	37.9	45	41	3	7
Normal to Moderate DM	16	7.10	9.4	762	13.4	34.7	49.1	18.9	38.4	45	44	4	6
Extreme DM	10	7.58	9.9	812	13.7	36.8	48.8	18.3	37.5	43	49	3	4
SEM[3]		0.20	0.6	44	0.3	0.9	0.7	0.2	0.3	4	4	1	1
Sign[4]		NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS	NS

[1] Location, scoring and categorization of animals are as is stated in the Methods and Materials of Chapter VI. There are four age groups (14 to 15, 26 to 27, 38 to 39 and 62 to 63 mo) and two sexes (female and male) per breed group. Each animal was restrained in a cattle squeeze and 7 mL of jugular blood were drawn into a Vacutainer containing EDTA. Blood collection was completed within five h and all samples were stored at 4°C. All hematology determinations were done at the Western Veterinary Diagnostic Laboratory in Vancouver on a Coulter S-Plus Counter. Samples were received by the Western Veterinary Diagnostic Laboratory the day following sample collection. Statistical analyses were as is stated in Chapter VI with sources of variance being phenotype, sex, phenotype x sex, age, phenotype x age and phenotype x sex.

[2] Number of animals per phenotype.

[3] Average standard error of least squares means.

[4] Significance: NS-not significant ($P>0.05$).

Abreviations are as follows: RBC=Red Blood Cell; WBC=White Blood Cell; Hgb=Hemoglobin; HCT=Hematocrit; MCV=Mean Corpuscular Volume; MCH=Mean Corpuscular Hemoglobin; MCHC=Mean Corpuscular Hemoglobin Concentration; POLYS=Polycytes; LYMPHS=Lymphocytes; MONOS=Monocytes; EOS=Eosinophils.